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CHARACTER EVOLUTION IN *ANAXAGOREA* (ANNONACEAE)[1]

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Anaxagorea is a critical genus for understanding morphological evolution in Annonaceae because it shares a variety of features with other Magnoliales that have been interpreted as primitive relative to other Annonaceae. We present a detailed discussion of morphological characters used in a combined morphological and molecular phylogenetic analysis of *Anaxagorea*, along with implications of the analysis for character evolution in the genus. In spite of a high level of homoplasy in stamen and leaf venation characters, their removal results in loss of resolution in the trees obtained. The distributions of characters on trees confirm assumptions that several distinctive similarities between *Anaxagorea* and other Magnoliales are primitive retentions (e.g., the presence of an adaxial plate of xylem in the midrib, nonpeltate stamen connectives, inner staminodes, and several leaf architectural characters). However, lateral extensions of the ''laminar'' stamens, though possibly ancestral in *Anaxagorea*, are convergent with those in other Magnoliales. A number of morphological synapomorphies have been identified for a clade containing most Central American species and another comprising all Asian species (e.g., conical bud shape and reduced inner petals for the Central American clade, and adaxial cuticular striations and capitate stigma shape for the Asian clade).

Key words: adaxial plate; *Anaxagorea*; Annonaceae; leaf architecture; morphology; stamens.

There have been several morphological and molecular cladistic studies of the angiosperm family Annonaceae, all of which have indicated that *Anaxagorea* is the sister group of the remaining taxa (Doyle and Le Thomas, 1994, 1996; van Zuilen, 1996; Doyle et al., 2000; Sauquet et al., 2003; Richardson et al., 2004). In the morphological analyses, the basal position of *Anaxagorea* was supported by its possession of several distinctive character states that also occur in other Magnoliales. However, these outgroup states are not seen in all species of *Anaxagorea*. In almost all such cases, it was assumed that the conditions shared with the outgroups were ancestral in *Anaxagorea*, and the genus (which was treated as a single taxon) was scored accordingly.

Some such characters concern the androecium. For example, most species of *Anaxagorea* possess inner staminodes, a trait also found in some other Magnoliales, namely Eupomatiaceae (*Eupomatia*), Degeneriaceae (*Degeneria*), and Himantandraceae (*Galbulimima*). However, two *Anaxagorea* species lack inner staminodes. Previous analyses scored the genus as having inner staminodes, and as a result this feature was one of several synapomorphies linking Annonaceae with *Eupomatia*, *Degeneria*, and *Galbulimima*, to the exclusion of Magnoliaceae, in the morphological and molecular analyses of Doyle and Endress (2000) and Sauquet et al. (2003). This scoring assumed that inner staminodes were originally present in *Anaxagorea* and were lost independently within *Anaxagorea* and in other Annonaceae. Another possibility is that *Anaxagorea* is paraphyletic and loss of inner staminodes is a synapomorphy of some *Anaxagorea* species and the rest of the family. Similarly, the stamens of most *Anaxagorea* species are more or less laminar, as in other Magnoliales, and thereby differ from the peltate stamens (with a cap-like connective extension) found in most other Annonaceae. However, some *Anaxagorea* species have stamens that approach the peltate type. Again, it was assumed that the laminar type was ancestral and deviations from it occurred independently in *Anaxagorea* and other Annonaceae.

Similar patterns are seen in leaf architecture and anatomy. Most species of *Anaxagorea* have brochidodromous leaf venation, but some are eucamptodromous. Eucamptodromous venation is also found in the majority of the other Annonaceae, whereas most other Magnoliales have a brochidodromous pattern, often of the festooned type (cf. Hickey and Wolfe, 1975). A feature found throughout other Magnoliales is an adaxial plate of xylem in the midrib. This plate seems to have been lost in Annonaceae, with the exception of most (though not all) species of *Anaxagorea*. Again, Doyle and Le Thomas (1996) assumed that the adaxial plate in *Anaxagorea* was a primitive feature retained from the ancestors of Annonaceae, which was lost independently within *Anaxagorea* and in other Annonaceae.

To test these assumptions requires an understanding of phylogenetic relationships in *Anaxagorea* and an examination of the variation within the genus in this framework. To address this problem and to evaluate biogeographic scenarios for the unique Asian-neotropical distribution of *Anaxagorea*, we conducted separate and combined analyses of morphological characters and plastid DNA sequences, the results of which are reported elsewhere (Scharaschkin and Doyle, 2005). The main objectives of the present article are (1) to provide a detailed justification of the morphological characters used in that study,

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TABLE 1. Morphological data matrix used in this study. a = 0/1, b = 0/2, c = 1/2, d = 1/3, e = 2/3, f = 0/1/2.

Taxa	1 2 3 4 5 6 7 12345678901234567890123456789012345678901234567890123456789012345
Myristicaceae	0100100020??20?00c0???a01???3??????????????????000000a111??00300?00????a00???
Degeneriaceae	0200010020??20?000????1110??0000??01?11?1?1?0000100000020000?10????02011?
Magnoliaceae	0200100000??2a?001????c0a0??1000??00????0?0?000010b1112020a00?00????0200??
Himantandraceae	0200000020??10?aa20???1010??3?????11?01?0?1?000001000012031??10????0?011?
Eupomatiaceae	010a0100200??10?0000???21a0??3?????01?11?0?0?00020100000220?0?10????00111?
Ambavia	00?00a0000af?c0?00???0???11???1?0??00????0?1?00100000100110100?1001010??111
Annickia	0??0000012???21?aa?120?1000??1100??00????3?1?000010211101213011111110 0ce211
Annona	00?000001113?21?001aca?1100??1100??00????3?0?0111212121 1?2c31??1aa100032e11
Artabotrys	10?001001111?21?00?020?1101??1101??00????3?0?001011ca21011120001aaa000d2311
Asimina	00?10000111b?01?00002a?1010??1000??00????3?0?0111212121121100001000001123 11
Cananga	00?000101213?01?00?020?0111??1101??00????0?0?01102100000221001111010011211 1
Isolona	00?10000111 2?01?00?0?0?2110??1?0???00????3?0?001111112001a102??101101033311
Malmea	00?000000011?20?000110?100a??1000??00????3?0?000010211112213010 11a010013211
Mkilua	0??0001001???20?00???0???00??1?00??00????3?0?111121????0121000?21a?001??311
Uvaria	10?010001101?21?aa1010?200a??1000??a0????3?0?0001110020ac2a00101aa101032311
Xylopia	00?010000101?2a?0000c0?0011??1101??11????3?0?11111100?002c200a0?a0100a1c311
Anaxagorea acuminata	0100010000b1100011020011110011001001010001 1100?0100010??2020100100000?2100
Anaxagorea allenii	0210000000011110101?200??1111111000010200100100?0100010??2020100100000?2100
Anaxagorea angustifolia	0??0011?00021??1001?200??1110110011010 11?20110????????????2020100100000??100
Anaxagorea borneensis	00?000001102101100 0?01111111021?01?0102002011000010001001212010010000 0?2100
Anaxagorea brachycarpa	0100010000000101001?200??1100110011011111102000?????????0120201001000 00??100
Anaxagorea brevipedicellata	0211100100010000011?200??1110110011 00???011100????????????2020100100000??100
Anaxagorea brevipes	020010000a021201111?200111110110011011211101000001000100120201001000000 2100
Anaxagorea crassipetala	0210000011021211101?200??1111111000010200100000?????????0120201001000000??100
Anaxagorea dolichocarpa	0200010001021210011020011 11a0110011011210ca11000010001001202010010000 0?2100
Anaxagorea floribunda	02001010011211a1111?200??11?0110001010100c???0??????????0?2020100?0?0????2100
Anaxagorea gigantophylla	0210100011021010011?200??1110110000011211101000001000100?2020100?0?0????2100
Anaxagorea guatemalensis	0210000001021011101?200111111111000010200200 00?????????????2020100100000?2100
Anaxagorea inundata	02100001000202a????????a0111011000101010011100?????????0121201 00?0?0????2100
Anaxagorea javanica	00?000001101001100 0?111110110c1?00?0102001001000010001001212010010000 0?2100
Anaxagorea luzonensis	00?000001102021100 0?1111101101100000 0???010010?????????0121201001000000 2100
Anaxagorea macrantha	0??0100001111c0?????200??1101110011011210101 00????????????2020100?????????00
Anaxagorea manausensis	02101100000b1c1?????200??11?0110001010200100 00?????????0120201 00?0?0????2100
Anaxagorea pachypetala	0210010011111111101?2001111?0110001010200101 00????????????2020100?0?0????2100
Anaxagorea panamensis	0110000000020c01101?200??1111111001010200010000001000100120201001000 00?2100
Anaxagorea petiolata	0210010000020ca1111?200111100110000010100101 10?????????0120201001000 00?2100
Anaxagorea phaeocarpa	0210110000a11211011020011110011000101121010110000010001001202010010000 0?2100
Anaxagorea prinoides	0210010000020201100020 0??11?0110001010000001100?0100010012020100100000 02100
Anaxagorea rheophytica	0200000100010001000 01????111011000101011010 1?0?????????0120201 00?0?0????2100
Anaxagorea rufa	0210001111021211111?200??1100110011011011100000001000100120201001000 00?2100
Anaxagorea silvatica	00?000011002010110001 00??011010000001011010010?????????????2020100100000 02100

in particular those showing variation in *Anaxagorea*, (2) to assess whether the similarities between *Anaxagorea* and the other Magnoliales were primitively retained or secondarily derived within *Anaxagorea*, and (3) to examine the evolution of morphological characters, in particular those pertaining to leaf architecture and stamen morphology, and their utility in determining phylogenetic relationships in the genus, using trees obtained from our combined morphological and molecular analysis.

MATERIALS AND METHODS

Taxon sampling—The 25 *Anaxagorea* species in our data set represent all currently recognized species of the genus except *A. radiata*, which may actually belong to *A. javanica* (Maas and Westra, 1984, 1985; Maas et al., 1986; Berry et al., 1999; Scharaschkin and Doyle, 2005). The 16 outgroups include the other families of Magnoliales, namely Myristicaceae, Magnoliaceae, Himantandraceae (*Galbulimima*), Degeneriaceae (*Degeneria*), and Eupomatiaceae (*Eupomatia*), and the same exemplars of the principal clades making up the rest of the Annonaceae that were included in a broad-scale analysis of Magnoliales by Sauquet et al. (2003) (Tables 1 and 2). Doyle and Le Thomas (1996) treated *Annona* and *Asimina* as members of the ''*Annona* group'' (*Annona*, *Anonidium*, *Raimondia*, *Rollinia*, and *Rolliniopsis*) and the ''*Asimina* group'' (*Asimina* and *Deeringothamnus*). Because most other members of the *Annona* group are probably nested within *Annona*, which was therefore used to score most characters, and because *Deeringothamnus* does not differ from *Asimina* in characters used in this analysis, we replaced these groups with *Annona* and *Asimina*, respectively. Recent molecular analyses indicate that *Anonidium* is related to *Neostenanthera* rather than *Annona* (Richardson et al., 2004), but its exclusion would affect scoring of only one character in the present data set (raphe shape: see below Results and Discussion, Seeds).

Morphological characters—Definitions of characters used in Scharaschkin and Doyle (2005), their relation to characters used in previous analyses, and sources of information are presented in the Appendix. As a starting point, we used all those characters of Doyle and Le Thomas (1996) that vary in the Annonaceae with the present taxon sampling, even if they did not vary in *Anaxagorea*, because of lack of consistent criteria for eliminating some such characters but not others and their potential value in determining the monophyly of *Anaxagorea*, reconstructing outgroup relationships, and understanding features of *Anaxagorea* in the context of morphological evolution in the family. Inferences concerning the evolution of characters in other Annonaceae that differ from results of Doyle and Le Thomas (1996) are often questionable because of our reduced taxon sampling, but they are mentioned to call atten-

TABLE 2. Voucher information for taxa examined for midrib histology, leaf architecture, stamen morphology, receptacle morphology, and seed coat anatomy.

Species	Midrib histology	Leaf X-ray	Stamen morphology	Receptacle morphology	Seed coat anatomy
Annonaceae					
Ambavia gerrardii (Baill.) Le Thomas	Rahajasoa et al. 902 (MO)	X	X	X	X
Anaxagorea acuminata (Dunal) A. DC	Molino 1350 (U)	Meier & Speckmaier 4326 (U)	Steyermark 89986 (U)	X	Steyermark & Espinoza 105877 (U)
Anaxagorea allenii R.E. Fr.	Galdames et al. 1266 (U)	Galdames et al. 1266 (U)	Croat 25137 (U)	Quinones 9501 (U)	Mosalve 1077 (U)
Anaxagorea angustifolia Timmerman	X	X	Pires 856 (U)	X	
Anaxagorea borneensis (Becc.) James Sincl.	Church et al. 771 (L)	Church et al. 771 (L)	Clemens et al. 20570 (U)	Church et al. 725 (L)	Ridsdale 467 (L)
Anaxagorea brachycarpa Becc.	Maas 6685 (U)	Maas 6685 (U)	Maas 6685 (U)	Nascimento 718 (U)	X
Anaxagorea brevipedicellata Timmerman	Lescure 214 (U)	de Granville B4919 (U)	Campbell et al. 22258 (U)	X	X
Anaxagorea brevipes Benth.	Clark et al. 7240 (U)	Gaunchez 291 (U)	Maas et al. 6362 (U)	Jansen-Jacobs et al. 1511 (U)	Jansen-Jacobs et al. 1511 (U)
Anaxagorea crassipetala Hemsley	Palacios 2638 (U)	Palacios 5223 (U)	Cuatrecasas 21275 (U)	Palacios 5223 (U)	X
Anaxagorea dolichocarpa Sprague & Sandw.	Clarke 4198 (U)	Maas et al. 8755 (U)	Polak 25 (U)	Maas et al. 8778 (U)	Maas et al. 8778 (U)
Anaxagorea floribunda Timmerman	Vásquez & Jaramillo 14472 (MO)	Vásquez et al. 17776 (MO)	X	X	Vásquez et al. 17776 (MO)
Anaxagorea gigantophylla R.E. Fr.	Davidse & Miller 27484 (U)	Davidse & 27484 (U)	Maguire et al. 36774 (U)	X	Davidse & Miller 27484 (U)
Anaxagorea guatemalensis Standley	Hazlett et al. 8039 (U)	Hazlett et al. 8039 (U)	Proctor et al. 35927 (U)	X	Hazlett et al. 8039 (U)
Anaxagorea inundata Berry and Miller	Berry et al. 6512 (U)	Berry et al. 6512 (U)	Berry et al. 6512 (DAV)	Berry et al. 6512 (U)	Berry et al. 6512 (U)
Anaxagorea javanica Blume	Leopold & Saikeh SAN 82690 (L) Agam & Aban SAN 41631 (L)	Leopold & Saikeh SAN 82690 (L)	Sinclair 40651 (U) Elmer 21131 (U) Kostermans 14010 (U)	Maxwell 80–92 (L)	Ramsri 54 (L)
Anaxagorea luzonensis A. Gray	Ramos PNH 1873 (L)	Ramos PNH 1873 (L)	van Benthem 1382 (U)	Kramer and Nair 6312 (L)	Bhargawa et al. 6312 (L)
Anaxagorea macrantha R.E. Fr.	X	X	Maas et al. 6749 (U)	X	X
Anaxagorea manausensis Timmerman	Vásquez & Criollo 5754 (U)	Vásquez & Criollo 5754 (U)	Miralha 301 (U)	Pipoly et al. 12195 (U)	Vásquez & Criollo 5754 (U)
Anaxagorea pachypetala (Diels) R.E. Fr.	Shunke, 6281 (MO)	Shunke 6281 (MO)	Tessmann 4393 (U)	X	Shunke 6281 (MO)
Anaxagorea panamensis Standley	Folsom & Collins 1699 (MO)	Croat 11158 (MO)	Croat 11158 (U)	Schmalzel 1130 (MO)	Folsom & Collins 1699 (MO)
Anaxagorea petiolata R.E. Fr.	Sanoja & Fernandez 3181 (U)	Sanoja & Fernandez 3181 (U)	Pinkus 172 (U)	Hernandez 462 (U)	Hernandez 462 (U)
Anaxagorea phaeocarpa Mart.	Daly 5994 (U)	Daly 5994 (U)	Schumke 4625 (U)	Diaz et al. 25 (U)	Maas et al. 8529 (U)
Anaxagorea prinoides (Dunal) A. DC.	de Granville 755 (U)	de Granville 755 (U)	de Granville B4529 (U)	Rosa and Vilar 3125 (U)	Plowman et al. 9416 (U)
Anaxagorea rheophytica Maas and Westra	Liesner 15879 (U)	Liesner 15879 (U)	X	Liesner 15879 (U)	Liesner 15879 (U)
Anaxagorea rufa Timmerman	Nee 30570 (U)	Nee 30570 (U)	Schultes & Caberar 14464 (U)	Anderson 13371 (U)	Nee 30570 (U)
Anaxagorea silvatica R.E. Fr.	Kolmann et al. 1768 (U)	Kolmann et al. 1768 (U)	Maas et al. 8836 (U)	X	Kolmann et al. 278 (U)
Annona montana Macfad.	Proctor 19812 (DAV)	Proctor 19812 (DAV)	van Andel 696 (U)	X	X
Artabotrys (1) *monteiroae* Oliv. (2) *hexapetalus* (L. f.) Bhandari	(1) Lovett & Congdon 2886 (P)	X	(2) Sandwyck 76GR00015 (U)	X	X
Asimina triloba (L.) Dunal	Fuller 16708 (DAV)	Fuller 16708 (DAV)	Morawetz 164 (U)	X	X
Cananga odorata (Lam.) Hook. f. & Thomson	Zanni et al. 16873 (NY)	Zanni et al. 16873 (NY)	X	X	X
Isolona campanulata Engl. & Diels	X	X	Sandwyck 6190 (U)	X	X

TABLE 2. Continued.

Species	Midrib histology	Leaf X-ray	Stamen morphology	Receptacle morphology	Seed coat anatomy
Mkilua fragrans Verdc.	X	X	Maas et al. 91GR01649 (U)	X	X
Xylopia peruviana R.E. Fr.	X	X	Maas et al. 6662 (U)	X	X
Degeneriaceae					
Degeneria vitiensis I. Bailey & A.C. Sm.	Webster & Hildreth 14101 (DAV)	Webster & Hildreth 14101 (DAV)	X	X	X
Eupomatiaceae					
Eupomatia laurina R. Br.	Webster & Tracey 18944 (DAV)	Webster & Tracey 18944 (DAV)	X	X	X
Himantandraceae					
Galbulimima belgraveana (F. Muell.) Sprague	Gray 1010 (MO)	Gray 1010 (MO)	X	X	X
Magnoliaceae					
Magnolia grandiflora L.	Israel 6 (DAV)	Israel 6 (DAV)	X	X	X
Myristicaceae					
Mauloutchia humblotii (H. Perrier) Capuron	Schatz & Modeste 2876 (MO)	Schatz & Modeste 2876 (MO)	X	X	X

tion to problems to be addressed in future broader-scale analyses. Second, we included characters from an analysis of basal angiosperms by Doyle and Endress (2000) that were potentially informative with the taxon sampling in this study. Additional characters, in particular those found to vary within *Anaxagorea*, based on our own observations and a literature search, were then added to the data set. Maas and Westra (1984, 1985), Maas et al. (1986), and Berry et al. (1999) were consulted extensively for *Anaxagorea* and are not cited individually. Other references used are mentioned for specific characters. Overall, 42 characters were taken directly from other studies, seven previously defined characters were modified, and 26 characters were newly constructed.

We made an effort to define characters that were not logically correlated and seemed to vary independently across taxa. For some character systems, such as leaf architecture, many other characters might be formulated, but most of these would be correlated with existing characters, and their inclusion would lead to overweighting of what are really single characters and might therefore result in spurious relationships or misleadingly high support for clades.

Approximately 10 characters are more or less quantitative. Use of such characters in phylogenetic analysis has been criticized by some authors (e.g., Stevens, 1991) but defended by others based on simulation studies and other empirical evidence (Thiele, 1993; Poe and Wiens, 2000). In an attempt to define systematically valid states in quantitative characters, we plotted data in histograms and looked for apparent natural breaks in the distribution of measures.

A problem frequently encountered in our study, as in many others that mix characters from broader-scale analyses with new characters that appear to be useful at the species level, is that many of the latter characters are excessively variable at higher levels. We found this was especially true for some quantitative characters. Also, in several quantitative characters, we were able to recognize breaks between states in *Anaxagorea*, but the same breaks did not appear to be valid in outgroup taxa. In such cases, we scored *Anaxagorea* for these characters but scored either all outgroups or other families of Magnoliales as unknown.

The resulting data set consists of 75 parsimony-informative characters, of which 49 are binary and 26 are multistate (Table 1). Five quantitative characters that were ordered in Doyle and Le Thomas (1996) were retained as such, while all the remaining characters are unordered. Variation in characters within taxa is scored as uncertainty (e.g., 0/1).

Leaves from all available species of *Anaxagorea* were sectioned to examine the midrib anatomy in detail, as were leaves of the outgroups, to verify previously published observations (Table 2). Leaves from herbarium specimens were soaked in water overnight. They were then freehand sectioned and stained with phloroglucinol for 1–2 min before examination under a light microscope.

Leaves from herbarium specimens were X-rayed using a cabinet-style analytical X-ray machine at the School of Veterinary Medicine, University of California, Davis. The basic procedure follows Wing (1992); the specific settings used were 11–12 kV, 3 mA, tube distance = 50.8 cm with a 2.5-min exposure time. X-radiographs were then examined under a dissecting scope for characters such as variation in higher-order venation. Voucher information for specimens X-rayed is listed in Table 2.

Stamen and staminode morphology was examined in detail using both light microscopy and scanning electron microscopy (SEM). Voucher information for specimens examined using SEM is listed in Table 2.

Morphology of the floral receptacle was studied by making longitudinal sections of preserved and boiled herbarium material. Mesotestal fibers were examined by making freehand sections of the seed coat with a razor blade and observing the fiber orientation under a dissecting microscope. A list of species sectioned and vouchers used to examine these characters is presented in Table 2.

Trees—Trees used in this study are derived from Scharaschkin and Doyle (2005). The molecular data consisted of DNA sequences from the *atpB-rbcL*, *psbA-trnH*, and *trnL-trnF* spacer regions and the *trnL* intron. In the analyses considered here we constrained outgroup relationships to the topology found by Sauquet et al. (2003), which is consistent with the arrangement of Annonaceae in Richardson et al. (2004). Relationships within *Anaxagorea* were unresolved in the strict consensus of more than 50 000 most parsimonious trees derived from the molecular data, but the use of agreement subtrees (Finden and Gordon, 1985) showed that relationships amongst a subset of taxa remained stable. These relationships included the nested position of two clades that contain all Asian and most Central American species, a result that was critical for biogeographic scenarios. Similarly, only a few clades occurred in the consensus of 60 most parsimonious trees based on morphology. However, relationships were better resolved in the four trees found in the combined analysis (Fig. 1a). The rooting of these trees is identical; the differences amongst them lie in the placement of the South American species *A. pachypetala* and the positions of *A. floribunda*, *A. macrantha*, *A. rufa*, and *A. phaeocarpa* in a large clade of South American taxa. This large clade, which contains the most recent common ancestor of *A. floribunda*, *A. acuminata*, and

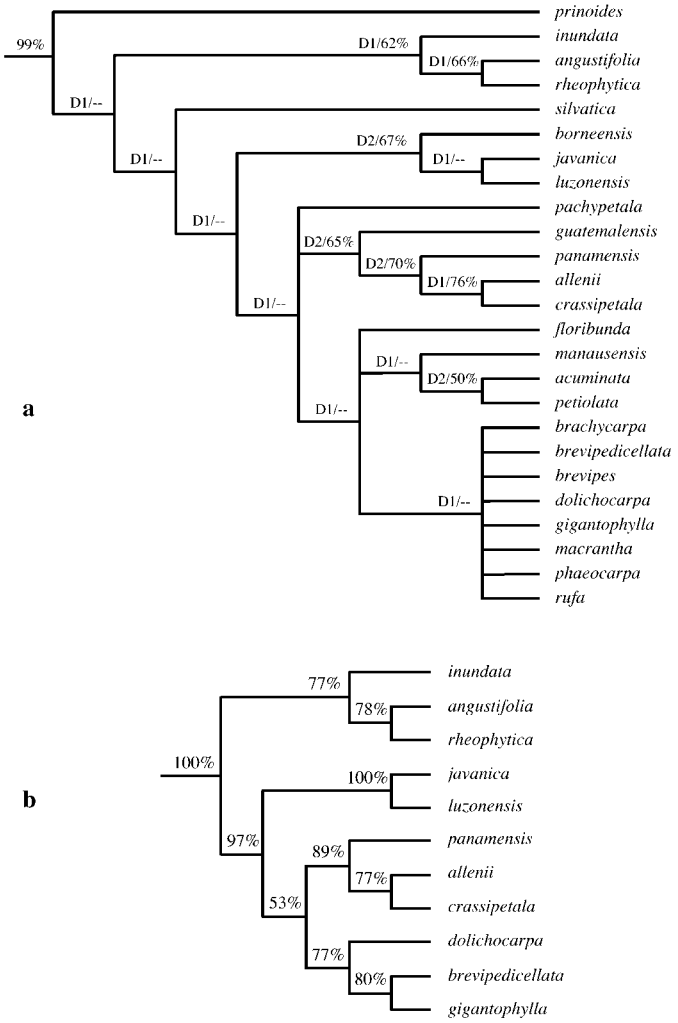

Fig. 1. Relationships in *Anaxagorea* inferred from parsimony analyses of combined morphological and molecular data (Scharaschkin and Doyle, 2005). (a) Strict consensus of four most parsimonious trees from an analysis of all species, with bootstrap and decay values, and (b) most parsimonious tree from an analysis of the species found in an agreement subtree based on the molecular data, with bootstrap values from an analysis of the combined data for this subset of species.

A. dolichocarpa and all of its descendants, will be referred to as the ''core South American clade.'' In analyses of all taxa, bootstrap and decay values within *Anaxagorea* were generally low (Fig. 1a). However, bootstrap values from an analysis of just those taxa found in one of the molecular agreement subtrees were much higher (Fig. 1b), suggesting there is a fairly robust ''backbone'' of relationships in the genus.

Anaxagorea was paraphyletic in some trees based on morphological data when outgroup relationships were not constrained, with *A. rheophytica*, *A. silvatica*, and the Asian species linked with other Annonaceae, based in part on loss of the adaxial plate in the midrib and inner staminodes, but monophyletic in other trees, and it was monophyletic in all trees when outgroup relationships were constrained. The monophyly of *Anaxagorea* was supported with high bootstrap values in both molecular and combined analyses (Fig. 1; Scharaschkin and Doyle, 2005).

We examined morphological evolution on two of the four trees produced by the combined analyses, which differ only in the placement of *A. pachypetala*, using MacClade (Maddison and Maddison, 2001). It is the variation in position of this species that has the greatest effect on inferred patterns of character evolution. These two trees will be referred to throughout as tree A and tree B. In tree A, *A. pachypetala* is the sister group of the core South American clade alone, whereas in tree B it is the sister group of the core South American clade plus the Central American clade. The other two most parsimonious trees differ in the arrangement of species in a subgroup of the core South American clade and therefore have less effect on evolutionary scenarios across the genus. The two trees used were chosen because they contain the only relationship in this clade (of *A. brevipedicellata* and *A. gigantophylla*) with substantial bootstrap support (Scharaschkin and Doyle, 2005).

The practice of reconstructing morphological evolution by optimizing characters on a tree based wholly or in part on morphological data has been criticized as involving circular reasoning (e.g., Hedges and Maxson, 1996). However, the concept that such reasoning is circular has itself been rejected on logical grounds (e.g., Lee, 1997; de Queiroz, 2000). Any such tree is based not only on the character whose evolution is being discussed at a given time, but also on all the other characters included in the analysis, which together provide a best available estimate of phylogenetic relationships. Even if the circularity argument is accepted, it would only require removing one character at a time from the analysis, not all morphological characters. In any case, the alternative procedure of optimizing morphological characters on a molecular tree would be a futile exercise in the present study, because well-resolved trees were obtained only by combining molecular and morphological data.

RESULTS AND DISCUSSION

Although definitions of characters and their states are presented in the Appendix, most of the practical and conceptual problems encountered and the arguments used in defining characters are treated in this section, because we view our analysis of morphological characters as one of the main results of this study. Results and discussion for sets of related characters are treated together in order to avoid unnecessary duplication.

Midrib histology—Midrib histology (character 2) is an aspect of leaf anatomy that has drawn much attention in Magnoliales, as most families of the order differ from other ''basal'' angiosperms in possessing an adaxial plate of xylem in the midrib. This feature was inferred to be a synapomorphy of Magnoliales by Doyle and Endress (2000). However, the adaxial plate is absent in most Annonaceae, with the conspicuous exception of some, though not all, *Anaxagorea* species (Fig. 2a–f). Doyle and Le Thomas (1996) scored the *Annona* group as 0/1 based on what they interpreted as an adaxial plate in *Annona* in fig. 23 of van Setten and Koek-Noorman (1986). However, this structure was labeled as sclerenchyma, and our observations on several *Annona* species confirm this interpretation, so we have rescored *Annona* as lacking an adaxial plate.

We subdivided the character state ''arc plus adaxial plate'' used by Doyle and Le Thomas (1996) into two states, because in some *Anaxagorea* species the adaxial plate of xylem forms a continuous layer fused with the arc of xylem, whereas in others it appears separated from the xylem arc (Fig. 2; contrast c and f). Doyle and Le Thomas (1996) recognized a third state (complex) with a possible modified adaxial plate in several taxa, including *Unonopsis* (van Setten and Koek-Noorman, 1986) and *Fusaea* (Chatrou and He, 1999), but these taxa are not represented in the present data set.

In *Anaxagorea*, some species have elaborate protuberances on the adaxial surface of the adaxial plate, whereas others have very simple plates or plates with inconspicuous projections, as in other Magnoliales (Fig. 2, contrast e and f). This was coded as an additional character (3), for which those taxa lacking a plate were scored as unknown (?).

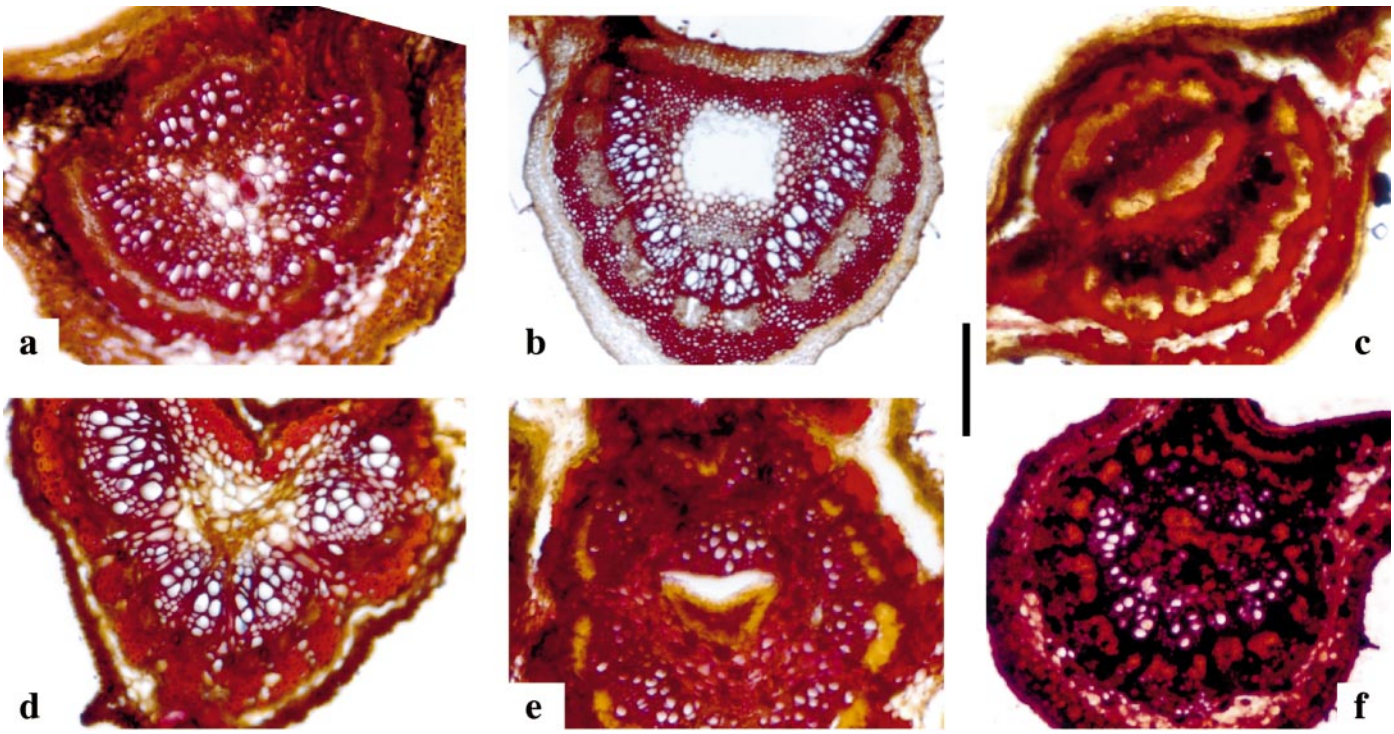

Fig. 2. Midrib anatomy. (a) *Galbulimima* (Himantandraceae): arc plus fused plate, protuberances absent/inconspicuous. (b) *Asimina* (Annonaceae): simple arc. (c) *Anaxagorea brachycarpa*: arc plus unfused plate, protuberances absent/inconspicuous. (d) *A. borneensis*: simple arc. (e) *A. crassipetala*: arc plus fused plate, prominent protuberances. (f) *A. rheophytica*: arc plus fused plate, protuberances absent/inconspicuous. Scale bar = 200 μm.

Serial sections were made along the length of the midrib to assess whether the degree of fusion changed as a function of distance from the petiole. It was easiest to observe the adaxial plate in the lower half of the blade, although if a distinct plate was present in this area, it usually continued as a recognizable structure through the rest of the blade. The only exception to this observation was in *Eupomatia*, in which the adaxial plate was actually more clearly visible in the middle to upper third of the blade. Sugiyama (1976a, b, 1979) did an extensive study on the node-leaf vascular anatomy in Magnoliales, including *Eupomatia*, and we consulted her observations closely in order to understand midrib histology. As a result of our observations on *Eupomatia*, which Sugiyama (1976b, 1979) described as having an adaxial plate, all those taxa that we had scored as lacking an adaxial plate were resectioned throughout the length of the blade to rule out the presence of an adaxial plate. Sections were also made through the petiole, as those taxa having an adaxial plate also have additional vascular bundles adaxial to the main arc of petiole bundles. Since our observations on the vascular bundle arrangement in the petiole were consistently correlated with status of the adaxial plate in the blade, we did not treat petiole anatomy as an additional character.

The presence of an adaxial plate has been cited as support for the basal position of *Anaxagorea* (Doyle and Le Thomas, 1996). This assumes that presence of this feature is ancestral in *Anaxagorea* and its absence in some species is due to loss. The members of the genus that lack an adaxial plate are the Asian clade (*A. borneensis*, *A. javanica*, and *A. luzonensis*) and the Brazilian species *A. silvatica*. This has been considered evidence that the Asian species and *A. silvatica* may be related (Maas and Westra, 1984). Maas et al. (1986) claimed that *A. rheophytica* also lacks an adaxial plate, but our observations clearly show that a plate is present (Fig. 2f).

The phylogenetic results support the assumption that the presence of an adaxial plate is ancestral in *Anaxagorea* and that the plate was lost independently within *Anaxagorea* and in the remaining Annonaceae (Fig. 3a). The strong bootstrap support for *Anaxagorea* (Fig. 1) rules out the possibility that the genus is paraphyletic, with the species without a plate linked with other Annonaceae (as in some morphological trees with outgroups unconstrained; Scharaschkin and Doyle, 2005). Because the Asian clade and *A. silvatica* form two adjacent lines rather than a clade (it is two steps less parsimonious to link the two groups), it is equivocal whether the plate was lost independently in these two lines or was lost once in their common ancestor and reappeared in the combined Central American and core South American clade. Most *Anaxagorea* species have an adaxial plate fused with the vascular arc, and this is homologous with the situation seen in the other Magnoliales. An unfused plate arose independently in three species.

The outgroups to the Annonaceae lack conspicuous protuberances on the adaxial plate; the presence of prominent protuberances is either a synapomorphy of *Anaxagorea* or a synapomorphy of the whole family that was lost in other members along with the adaxial plate (Fig. 3b). However, there were multiple losses or reductions of the protuberances within *Anaxagorea*.

Leaf architecture—Appearance of the midrib on the surface of the leaf (character 4) was defined as having two states, because variation within taxa necessitated treating concave and flat together as one state as opposed to convex, whereas for the secondary veins (character 5) flat and convex were usually

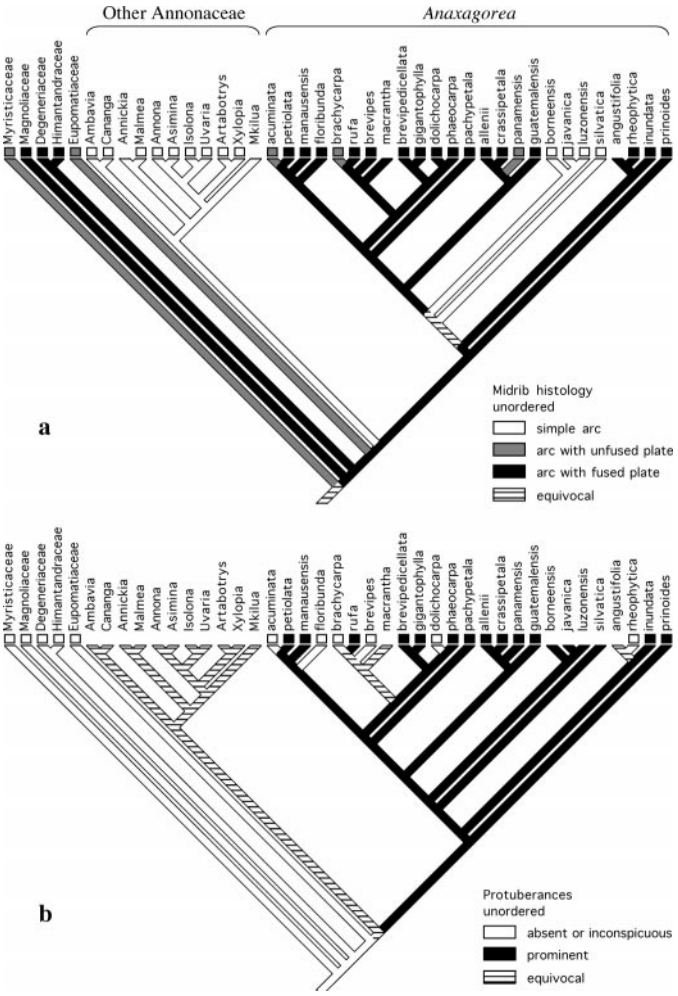

Fig. 3. Tree A, showing inferred evolution of midrib histology (a) and protuberances on the adaxial plate (b) in *Anaxagorea* and outgroups.

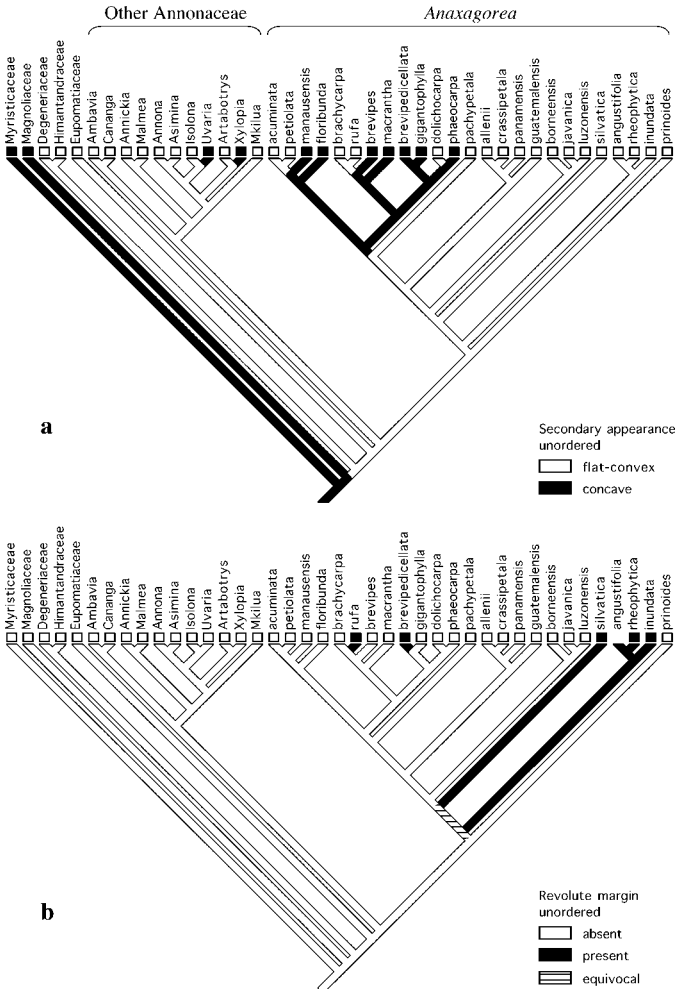

Fig. 4. Tree A, showing inferred evolution of the appearance of secondary veins on the upper leaf surface (a) and revolute leaf margins (b) in *Anaxagorea* and outgroups.

associated. The midrib was originally concave-flat in Magnoliales and became raised in *A. brevipedicellata* and two lines in other Annonaceae (*Asimina*, *Isolona*), as well as in several genera not included in the present data set (cf. Doyle and Le Thomas, 1996), without uniting any of these taxa. The secondary veins are primitively convex-flat in Myristicaceae and Magnoliaceae, but the four other families of Magnoliales are united by a shift to flat or convex secondaries (Fig. 4a). A shift back to concave secondaries is a synapomorphy of the core South American clade, with three reversals to flat or convex. These observations clearly show that the topography of the midrib and that of the secondaries are not correlated.

We treated the shape of the leaf base as two separate characters, presence of a decurrent base (character 6) and symmetry of the base (character 7), because it seemed that these two aspects of shape are not correlated. Both characters are very homoplastic, but it is worth noting that the ancestral condition in *Anaxagorea* is a nondecurrent, symmetrical leaf base, as in most other Annonaceae.

Another potentially informative feature of some *Anaxagorea* species is distinctly revolute leaf margins (character 8). Revolute margins arose independently in *A. rufa*, *A. brevipedicellata*, and either once or twice in *A. silvatica* and the *A. rheophytica-A. inundata* clade (Fig. 4b).

Doyle and Le Thomas (1996) treated secondary venation as one character. However, close examination of the variation in *Anaxagorea* revealed that the type of venation (character 9), as defined by Hickey (1973), and the degree of vein curvature (character 10) are not always correlated. Doyle and Le Thomas (1996) concluded that their results supported the view that there was ''increasing regularity of successively higher vein orders'' (increase in ''rank''; Hickey and Wolfe, 1975) within the family. Most *Anaxagorea* species have relatively irregular, ''low-rank'' brochidodromous venation (Fig. 5a), but some species have a pattern that we describe here as approaching eucamptodromous (Fig. 5b), i.e., with the secondary veins thinning as they approach the leaf margin and some of them not forming distinct brochidodromous loops. Many other Annonaceae show this transitional eucamptodromous venation, while some or all members of the other families of Magnoliales have pronounced festooned brochidodromous venation, with many successive secondary loops towards the margin of leaf (Fig. 5c).

The present results (Fig. 6a) support the hypothesis that brochidodromous venation is ancestral in *Anaxagorea*, and they indicate that the almost eucamptodromous condition arose independently in several lines. None of the Annonaceae in our

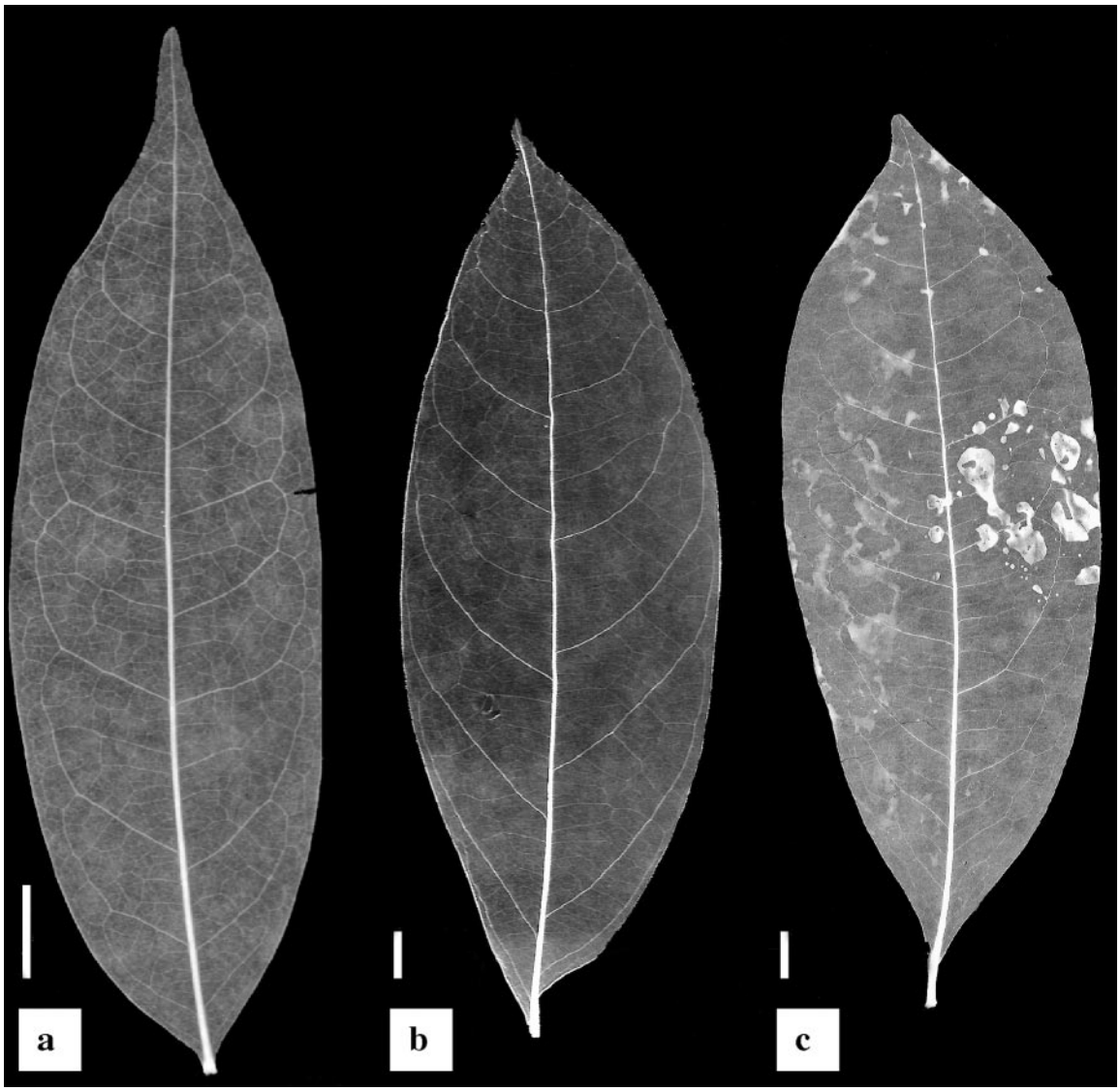

Fig. 5. Leaf architectural characters. (a) *Anaxagorea brachycarpa*: brochidodromous, straight secondary veins, branched intersecondaries, reticulate tertiary venation. (b) *A. borneensis*: approaching eucamptodromous, moderately curved secondary veins, intersecondaries absent or infrequent, weakly percurrent tertiary venation. (c) *Eupomatia laurina*: festooned brochidodromous, straight secondary veins, branched intersecondaries, reticulate tertiary venation. Scale bar = 1 cm.

data set have the festooned brochidodromous venation inferred to be basic in Magnoliales; the shift from festooned to strictly brochidodromous is a synapomorphy of the family. Depending on the placement of *A. pachypetala*, one shift to eucamptodromous venation could account for its presence in *A. silvatica*, the Asian species, and *A. pachypetala*, as in tree B (Fig. 6a); but if *A. pachypetala* is sister to the core South American clade only, as in tree A, the condition on the stem between the *A. angustifolia-A. inundata* clade and *A. pachypetala* is equivocal.

Optimization of curvature of the secondary veins (Fig. 6b) illustrates how this character varies independently of the type of secondary venation. In *Anaxagorea*, some species have straight secondary veins (Fig. 5a), while others have moderately curved secondaries (Fig. 5b). Moderately curved veins are also present in many other Annonaceae, but strongly curved veins are less common in the present taxon set (Fig. 6b). *Anaxagorea* retains the straight or recurved secondary veins of other Magnoliales as its reconstructed ancestral state. The change to moderately curved veins is a synapomorphy of the Asian, Central American, and core South American clades, although this is a rather homoplastic character, with several reversals to straight-recurved veins.

Secondary vein spacing (character 11) and angle (character 12) were considered too variable within taxa for inclusion in Doyle and Le Thomas (1996), but for the purposes of our finer-scale study such information appeared to be potentially informative. Irregularly spaced secondary veins (Fig. 5a, b) are ancestral in *Anaxagorea*, a symplesiomorphy with some of the outgroups to Annonaceae (Fig. 5c). Other Annonaceae are united by a shift to uniform spacing, although this result needs confirmation with broader taxon sampling. In most *Anaxagorea* species (Fig. 5b), the angle of the secondary veins decreases towards the base of the leaf, and this is the ancestral

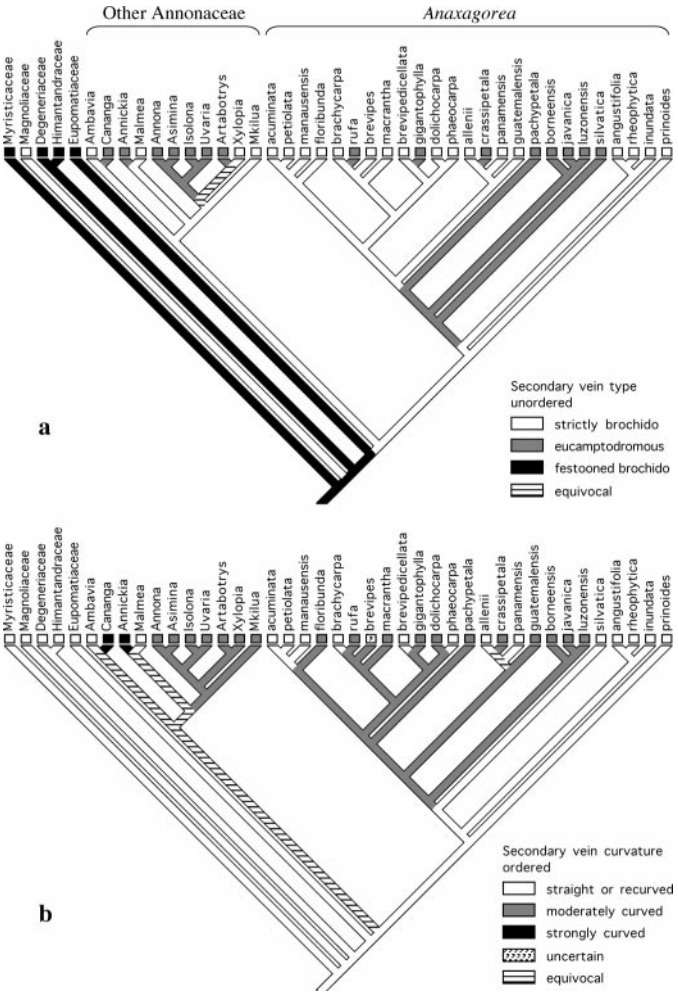

Fig. 6. Tree B (a), showing inferred evolution of secondary vein type, and tree A (b), showing inferred evolution of secondary vein curvature in *Anaxagorea* and outgroups.

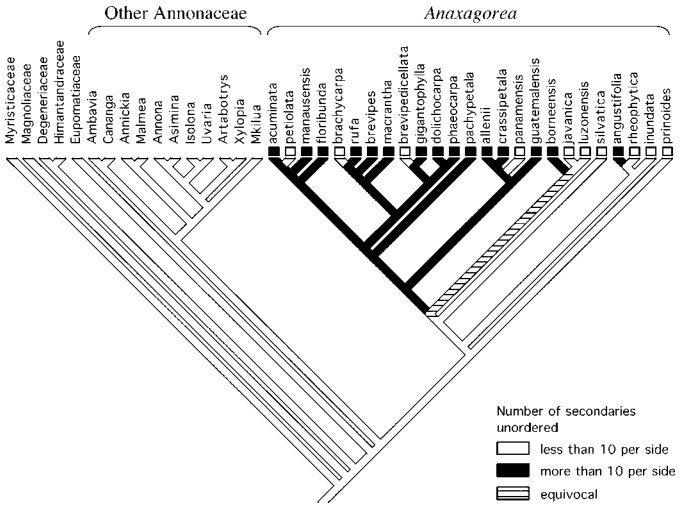

Fig. 7. Tree A, showing inferred evolution of number of secondary veins on each side of the leaf in *Anaxagorea* and outgroups.

state for the genus, with some lines independently changing to uniform or irregular.

The number of secondary veins (character 13) is another character not included in any previous study of Annonaceae, and in the present analysis it was recorded only in *Anaxagorea*, because it appeared to be too variable in the outgroups. Our counts were based on larger, mature leaves on a given branch, excluding small basal leaves and incompletely developed leaves. In plotting the average number of secondary veins on each side of the leaf, we found a clear break in the distribution, with most species having either more than 10 or fewer than 10 veins per side. The ancestral condition in *Anaxagorea* is less than 10 secondary veins per side (Fig. 7). The increase to more than 10 veins on each side is either a synapomorphy of the core South American clade and the Central American clade, with an independent origin in *A. borneensis* in the Asian clade, or a synapomorphy of all these groups, with a reversal in the Asian species *A. javanica* and *A. luzonensis*.

Intersecondary veins are quite common in *Anaxagorea*. The states vary independently of the type of secondary and tertiary venation, and we therefore treated them as a new character (14). Figure 5a shows an example of branched intersecondaries, while Fig. 5b shows a case in which intersecondaries are infrequent or absent. There is a high level of homoplasy in this character (Fig. 8a), but the ancestral condition in *Anaxagorea* is presence of intersecondaries that curve downward or are unbranched, which our results indicate is a retention of the same state in most other Annonaceae and Magnoliales.

The tertiary venation character (15) was taken from Doyle and Le Thomas (1996), with the following modifications: the development of intersecondary veins was treated as a separate character (as discussed earlier), and partially percurrent and strongly percurrent tertiaries were combined into one state, because this distinction was less clear with the taxon sampling in this study. In *Anaxagorea*, some species have reticulate tertiary venation (Fig. 5a), while others have weakly percurrent tertiaries (Fig. 5b). Our results confirm the view of Doyle and Le Thomas (1996) that Annonaceae originally had reticulate tertiary venation, and this is the ancestral condition in *Anaxagorea* (Fig. 8b). There were several shifts to percurrent tertiaries in other Annonaceae, and a similar advance occurred independently within *Anaxagorea*, uniting the Asian, Central American, and core South American clades, but with some reversals to reticulate tertiaries.

We examined several other leaf architectural characters but concluded they were too variable within taxa for inclusion in a phylogenetic study (e.g., leaf shape, leaf size, distance between margin of the leaf and secondary loops, and length-to-width ratio of intercostal areas).

It is significant that if leaf architectural characters are removed and the resulting combined data set is reanalyzed, there is an increase in the number of equally parsimonious trees produced—from four trees with the complete character set to 4839. A strict consensus of these 4839 trees shows a marked decrease in resolution in *Anaxagorea*. Only the Central American clade (with the same internal relationships) and the clade consisting of *A. angustifolia* and *A. rheophytica* are still present. This decrease in resolution is indicative of the utility of leaf architectural characters in phylogenetic studies. There is also a slight decrease in the consistency index and retention index after removal of leaf architectural characters, from CI = 0.695 to 0.692 and from RI = 0.705 to 0.700, but this change is probably too small to be meaningful.

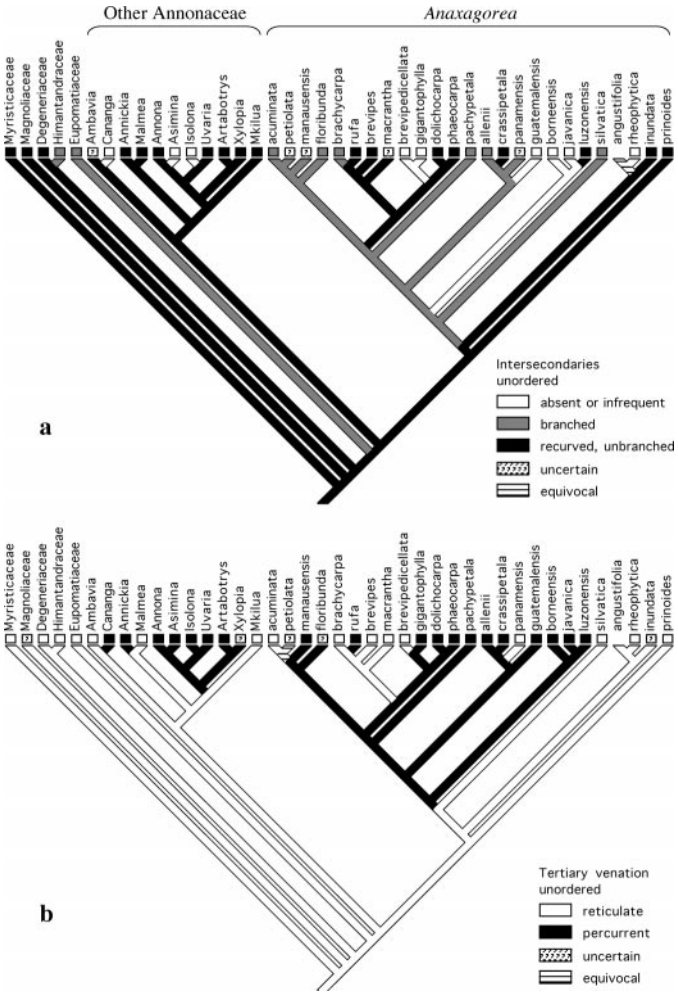

Fig. 8. Tree A, showing inferred evolution of intersecondary vein type (a) and tertiary venation (b) in *Anaxagorea* and outgroups.

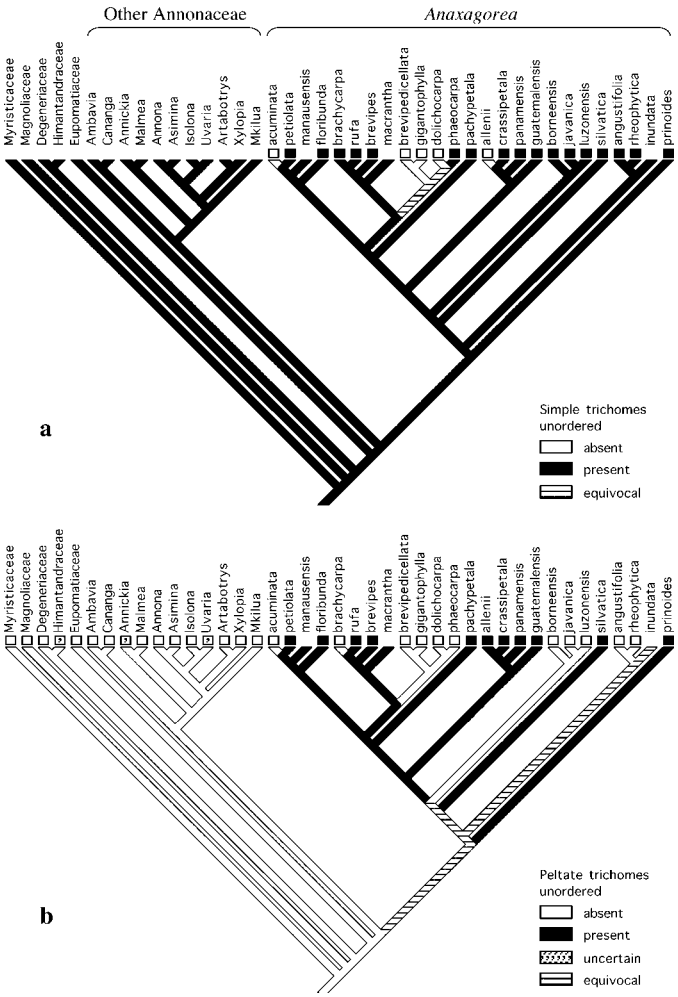

Fig. 9. Tree A, showing inferred evolution of simple trichomes (a) and peltate trichomes (b) in *Anaxagorea* and outgroups.

Leaf and wood anatomy—Doyle and Le Thomas (1996) treated foliar hairs as a single character with two states: (0) simple or absent, (1) stellate or peltate. However, Maas and Westra (1984), Koek-Noorman and Berendsen (1985), and Maas et al. (1986) described *Anaxagorea* as having simple, 2–4 branched (peltate), and stellate trichomes, with some species having only one type but others having more than one. For this reason, we have treated hairs as three separate characters. The presence of simple trichomes (character 16) is ancestral in *Anaxagorea*, and most species have simple hairs, except for a few losses (Fig. 9a). The ancestral state for peltate trichomes (character 17) is equivocal (Fig. 9b). Their absence is either a synapomorphy or a plesiomorphy of the Asian *Anaxagorea* species, but it is an unequivocal synapomorphy for one clade in the core South American group. Stellate trichomes (character 18) are ancestrally absent in *Anaxagorea*, and their origin is an important synapomorphy of the core South American clade, with only a single loss in *A. brachycarpa*.

Foliar sclereids (character 19) and epidermal crystals (character 20) are characters taken without modification from Doyle and Le Thomas (1996). Sclereids showed a high level of homoplasy in Annonaceae. Given their more extensive taxon sampling and their scoring of *Anaxagorea* as having astrosclereids, Doyle and Le Thomas (1996) concluded that astrosclereids were ancestrally present in Annonaceae and homologous with those in Myristicaceae and Magnoliaceae. This is not the case in the present study (Fig. 10a), because Myristicaceae and Magnoliaceae no longer appear to be closely related to Annonaceae, and our trees indicate that sclereids were originally absent in *Anaxagorea*. However, the situation in the remaining Annonaceae is uncertain because of our reduced taxon sampling and the lack of data for several taxa. Within *Anaxagorea*, origin of astrosclereids is an important synapomorphy uniting the Central American clade, *A. pachypetala*, and the core South American clade. The presence of druses is a shared feature of *Anaxagorea* and many other Annonaceae; however, not enough *Anaxagorea* species have been studied to make any conclusive remarks.

Distribution of oil cells in the leaf (character 21) was modified from Doyle and Le Thomas (1996) by the addition of a third state, (0) absent, since *A. borneensis* has no oil cells. In Doyle and Le Thomas (1996) the ancestral state in Annonaceae was equivocal, since the condition in *Anaxagorea* was mixed. Based on the present relationships, it appears that the presence of oil cells in the palisade parenchyma (or both the palisade and sponge parenchyma) is ancestral in both *Anaxagorea* and Annonaceae as a whole, with an equivocal number

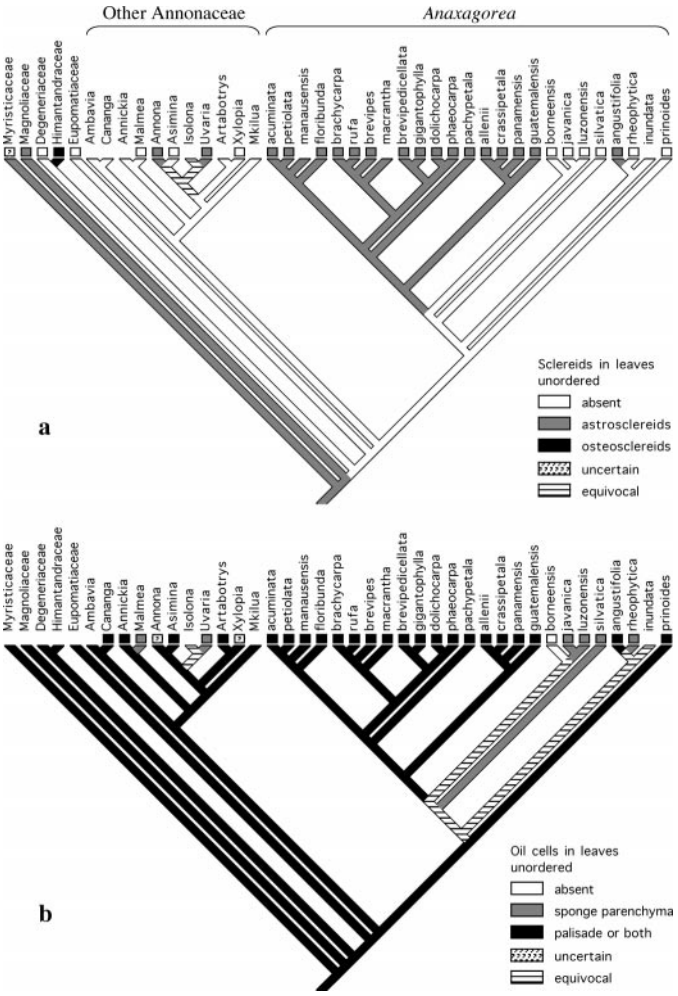

Fig. 10. Tree A, showing inferred evolution of sclereids (a) and oil cells in the leaves (b) in *Anaxagorea* and outgroups.

of restrictions of oil cells to the sponge parenchyma and a loss in *A. borneensis* (Fig. 10b).

The shape of anticlinal cell walls in unspecialized epidermal cells on the abaxial leaf surface (character 22) and the presence of striations on the adaxial surface (character 23) are two new characters added to this study, as fairly extensive information is available for *Anaxagorea* (Maas and Westra, 1984) and in the case of the anticlinal walls for other Annonaceae as well (Roth, 1981). Straight to slightly curved anticlinal walls and adaxial striations are important synapomorphies of the Asian clade, to which these states are restricted.

Our two wood anatomical characters were taken from Doyle and Le Thomas (1996), with the addition of data for *Anaxagorea* species. Vessel density (character 24) is not informative within *Anaxagorea*, as all species studied have a density of 10–40 vessels/mm^2, except for *A. inundata*, which straddles the limit between this state and <10 vessels/mm^2. Similarly, *A. inundata* is the only *Anaxagorea* species known to have narrow rays (character 25). Because of the presence of narrow rays in *A. inundata*, the lack of data for other near-basal species of *Anaxagorea*, and the scattered distribution of ray width in other Annonaceae, the ancestral condition in *Anaxagorea* is equivocal, rather than wide, as assumed by Doyle and Le Thomas (1996). As a result, wide rays may be either a synapomorphy or a symplesiomorphy for the Asian clade, the core South American clade, and *A. pachypetala*. The ancestral state for the family is also equivocal, as in Doyle and Le Thomas (1996). Both these characters are rather homoplastic for the family as a whole, as noted by Doyle and Le Thomas (1996).

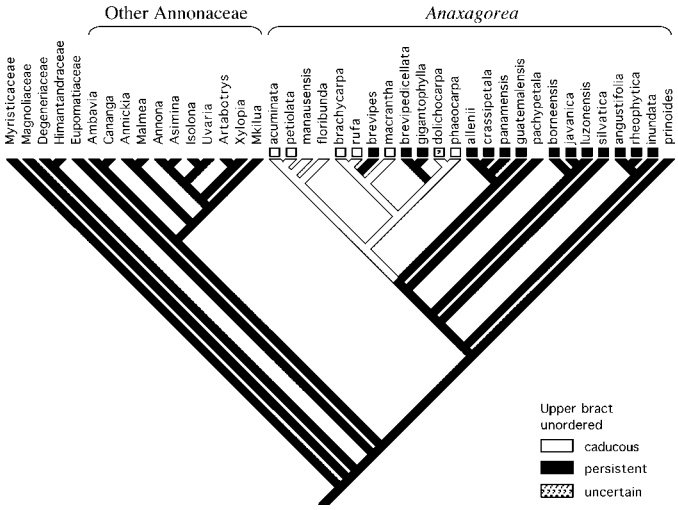

Fig. 11. Tree B, showing inferred evolution of persistence of the upper bract in *Anaxagorea* and outgroups.

Inflorescences—The inferred ancestral state for inflorescence position (character 26) in *Anaxagorea* is axillary, with terminal inflorescences originating independently in *A. silvatica* and the subgroup of the Asian clade consisting of *A. javanica* and *A. luzonensis*.

Annonaceae are often described as having either solitary flowers or cymes (rhipidia), but as discussed by Doyle and Le Thomas (1996) this distinction (character 27) is problematic. As pointed out by L. Chatrou (Utrecht University, personal communication), all Annonaceae except the *Cymbopetalum* group (Bocageeae: Johnson and Murray, 1995) have bracts on the pedicel that are capable of producing additional flowers from their axils and often do so occasionally even when they usually have solitary flowers. Hence Doyle and Le Thomas defined their character to distinguish taxa that usually have only one flower at a time from those that have several. As such, this character is more quantitative than qualitative. Under this definition, all *Anaxagorea* species bear flowers in cymes, but given the present sampling of Annonaceae, the ancestral condition in the family is equivocal, rather than cymes as concluded by Doyle and Le Thomas (1996). More extensive taxon sampling and quantitative evaluation of variation in this character are needed.

Persistence of the upper bract on the pedicel (character 28) is an informative character for *Anaxagorea*, in which persistent bracts are ancestral. In tree B, caducous bracts are a synapomorphy for the core South American clade, although a reversal to persistent bracts unites *A. brevipedicellata* and *A. gigantophylla* (Fig. 11). However, whether the transition to caducous bracts occurred at the base of this clade or below the node uniting it with *A. pachypetala* is equivocal in tree A, as the condition in *A. pachypetala* is not known.

Floral bud and corolla—Floral bud shape (character 29) varies greatly in the outgroups but was recognized by Maas

and Westra (1984) as potentially informative in *Anaxagorea*. Our results indicate that conical buds are an important synapomorphy of the Central American clade. The only other group in which buds became conical is *A. macrantha*, in the core South American clade.

The petal whorl character (30) is a combination of information from Doyle and Le Thomas (1996) and Doyle and Endress (2000), with the addition of the state ''inner whorl absent,'' found in *A. borneensis* and a variety of *A. javanica*, both Asian taxa. The presence of two whorls of petals is ancestral for *Anaxagorea*, as for Annonaceae as a whole, but the inner whorl was lost independently in *A. borneensis* and *A. javanica*.

All *Anaxagorea* species except *A. silvatica* have valvate petal estivation (character 31). With *A. silvatica* nested within *Anaxagorea*, valvate petals are ancestral in Annonaceae as a whole, as inferred by Doyle and Le Thomas (1996). Doyle and Le Thomas also concluded that the valvate state was a synapomorphy of the family, but because the sister group of Annonaceae appears to be *Eupomatia*, which has no petals, its point of origin is now equivocal.

Relative length of petals (character 32) was treated by Doyle and Le Thomas (1996) in terms of two characters, for inner and outer petals (their characters 34 and 35), in which the states were subequal vs. reduced or absent, but here the presence or absence of petal whorls has been taken into account in character 30. Some *Anaxagorea* species have inner petals that are conspicuously shorter than the outer petals, and this character was therefore introduced. The presence of shorter inner petals is a conspicuous synapomorphy of the Central American clade, along with conical bud shape, but the two characters do not appear to be intrinsically correlated, as *A. macrantha*, the only South American species in which buds became conical, does not have shorter inner petals.

Petal shape (character 33), as defined by Doyle and Le Thomas (1996), is not informative for relationships in *Anaxagorea*, in which all species have oval petals, and this aspect of petal morphology is symplesiomorphic with the rest of the family. However, the petals of all *Anaxagorea* species are unusual in being relatively fleshy. In many species the petals also have a conspicuous keel, but others have bowl-shaped petals without any protrusions.

Two separate characters for inner and outer petals were included in this study, because the presence or absence of a keel varies independently in the two whorls. Because they appear to be correlated with thick petals, these characters were not scored outside *Anaxagorea*. The keel on the outer petals (character 34) is a rather homoplastic character that arose independently in several lines and may have undergone one or two reversals to the ancestral condition of no keel (Fig. 12a). The keel on the inner petals (character 35) is equally homoplastic, but its presence is ancestral rather than derived. Reconstruction of its subsequent history depends on the position of *A. pachypetala*. In tree A, one loss of the keel accounts for its absence in *A. silvatica*, the Asian clade, and most species of the Central American clade (Fig. 12b), and its reappearance is a synapomorphy of *A. pachypetala* and the core South American clade. In tree B, with *A. pachypetala* interpolated between the Asian and Central American clades, it is equivocal whether the keel was lost several times or lost once and regained several times between the *A. angustifolia-A. inundata* clade and the core South American clade.

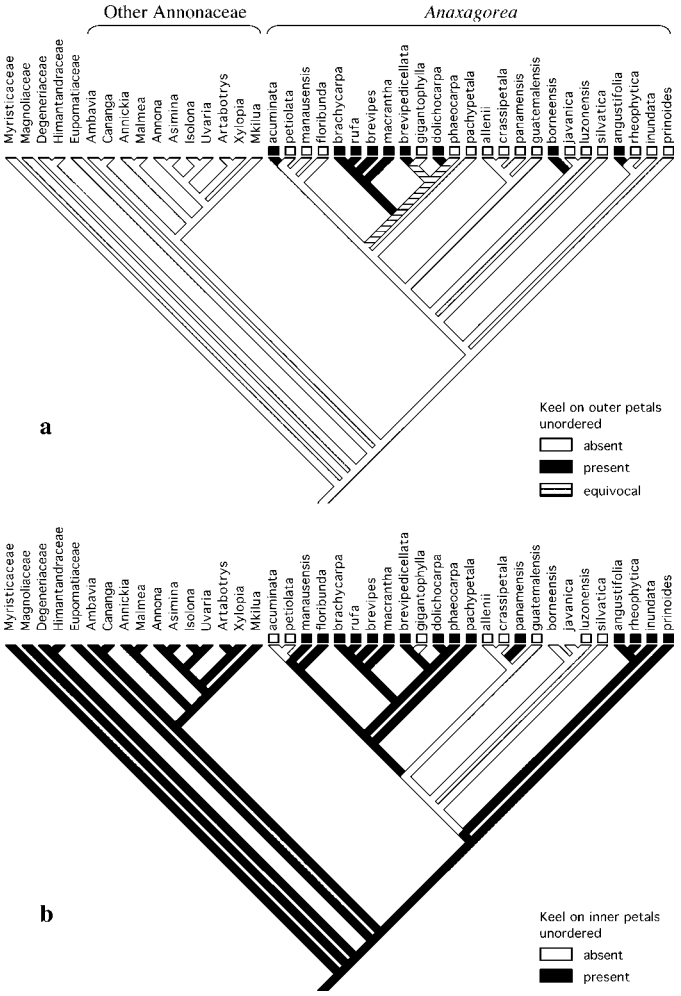

Fig. 12. Tree A, showing inferred evolution of a keel on the outer petals (a) and the inner petals (b) in *Anaxagorea* and outgroups.

Stamens and staminodes—Scoring of taxa for outer and inner staminodes (characters 36 and 37) follows Doyle and Le Thomas (1996), except that two *Anaxagorea* species (*A. brevipedicellata* and *A. luzonensis*) lack inner staminodes and were scored accordingly. Outer staminodes, which are absent in *Anaxagorea*, arose independently in three outgroup taxa (*Xylopia*, *Uvaria* in part, and *Galbulimima* = Himantandraceae). The inferred history of inner staminodes is quite different. Our results indicate that their absence in *A. brevipedicellata* and *A. luzonensis* was due to two independent losses within *Anaxagorea*. The presence of inner staminodes is therefore homologous in *Anaxagorea* and the closest outgroups of Annonaceae (*Eupomatia*, *Degeneria*, *Galbulimima*), and it provides important support for the sister group relationship of *Anaxagorea* to other Annonaceae, in which inner staminodes were lost, as assumed by Doyle and Le Thomas (1996).

Xylopia also has inner staminodes, but the tree implies that these arose independently. Because *Xylopia* and *Anaxagorea* were also distantly separated in previous analyses, we did not score *Xylopia* for subsequent inner staminode characters. To do so would run the risk of the Maddison effect (Maddison, 1994), where characteristics of a structure in one taxon affect the inferred ancestral state in another taxon in which the structure originated independently.

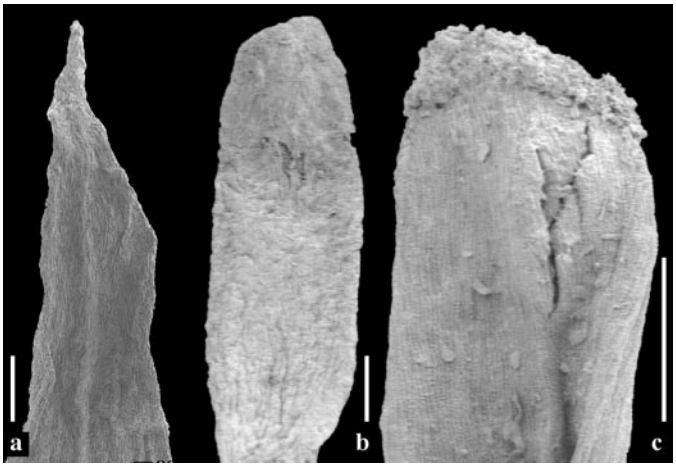

Fig. 13. Staminode morphology in *Anaxagorea*. (a) *A. prinoides*: pointed, lacking glandular areas. (b) *A. petiolata*: round, lacking glandular margin. (c) *A. brachycarpa*: round, glandular area present. Scale bar = 250 μm.

In *Anaxagorea*, some species have a large number of inner staminodes (character 38), and the distribution of numbers shows a clear break around 20, allowing the definition of two states. The presence of a large number of inner staminodes is a prominent synapomorphy of the clade containing the most recent common ancestor of *A. brachycarpa* and *A. phaeocarpa* and all its descendants, which makes up the larger part of the core South American group. The lack of homoplasy in this character illustrates the systematic value of such quantitative characters.

The shape of the tip of the inner staminodes (character 39) was a difficult character to break into states, because of the great amount of variation within taxa. A number of different options were examined; the current definition appears to be the best way to handle the polymorphisms, since taxa with truncate staminodes often have rounded ones too, but other taxa have strictly rounded staminodes. Some taxa have glandular areas on the edge of the inner staminodes (character 40), whereas others do not. Glandular areas, or mats of unicellular hairs secreting a sticky substance, have also been described in *Degeneria*, *Galbulimima*, and *Eupomatia* (Endress, 1984, 1994b; Endress and Hufford, 1989), and these were scored for this character. Figure 13 shows some of the diversity in the shape of the apex of the inner staminodes and the presence or absence of glandular areas in *Anaxagorea*.

Although one of the basal lines in *Anaxagorea*, *A. prinoides*, has pointed staminodes, it is most parsimonious to reconstruct the ancestral staminode shape in *Anaxagorea* as rounded, as in *Degeneria* and *Eupomatia* (Fig. 14a). A shift to generally truncate staminodes is a synapomorphy of the Asian, Central American, and core South American clades, with a few further modifications within the South American clade. Whether glandular areas on the inner staminodes are ancestral or derived in *Anaxagorea* is equivocal, as is their homology with the glandular areas in other Magnoliales. However, in the core South American clade, the reappearance of glandular areas provides an additional synapomorphy for the clade that was also united by an increased number of inner staminodes (Fig. 14b). These two characters are not completely correlated, as there are species elsewhere in the genus that have glandular areas but fewer inner staminodes. The role of these features in pollination is an interesting topic for future investigation.

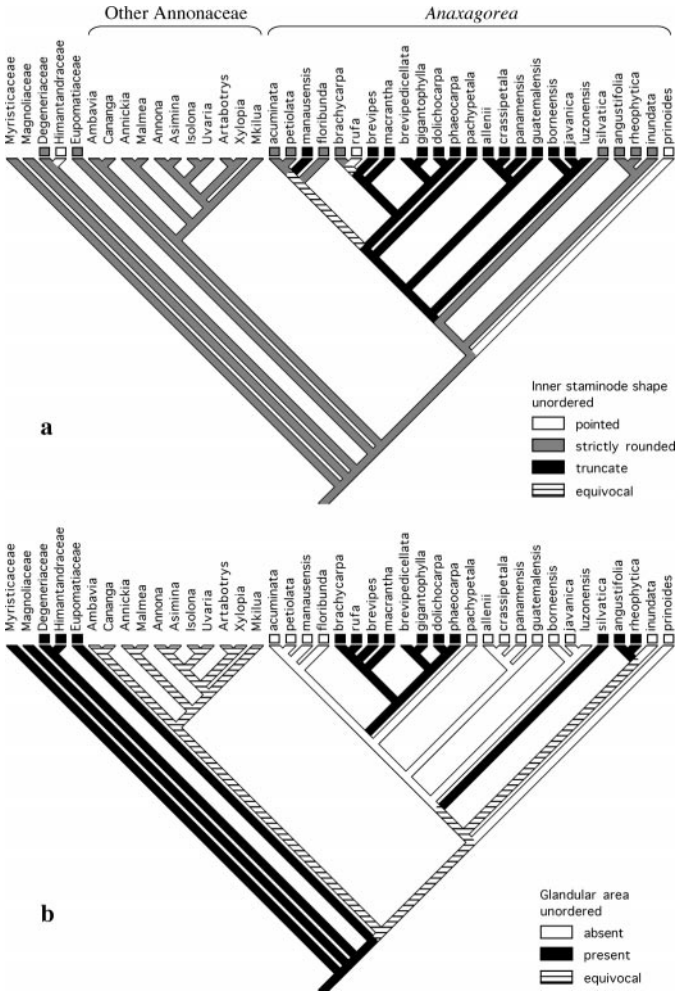

Fig. 14. Tree A, showing inferred evolution of inner staminode shape (a) and glandular areas on inner staminodes (b) in *Anaxagorea* and outgroups.

Information on stamen number in *Anaxagorea* (character 41) was available from the monograph of Maas and Westra (1984, 1985). A break in the distribution of numbers occurs at about 100. An increase in stamen number unites three species in the core South American clade (*A. brachycarpa*, *A. rufa*, and *A. brevipes*), but it occurred independently in *A. gigantophylla*, situated elsewhere in the same clade.

Three aspects of stamen form (Fig. 15) appear to vary independently and were therefore treated as separate characters. Although stamens in *Anaxagorea* are more or less laminar, resembling those of most other Magnoliales (Doyle and Le Thomas, 1996), they vary considerably in the shape of the apex (character 42), as well as in whether the apex of the connective is bent and if so, whether it is bent towards the thecae (abaxially) or away from them (adaxially) (character 43). The third character (44) accounts for whether the connective extends laterally beyond the thecae, resulting in the most strongly laminar stamens, which were considered primitive by Walker (1971). We introduced an additional character (45) to express the fact that in some *Anaxagorea* species those stamens close to the inner staminodes differ markedly from the outer stamens, whereas in other species there is no such differentiation.

The ancestral shape of the stamen apex in *Anaxagorea* was

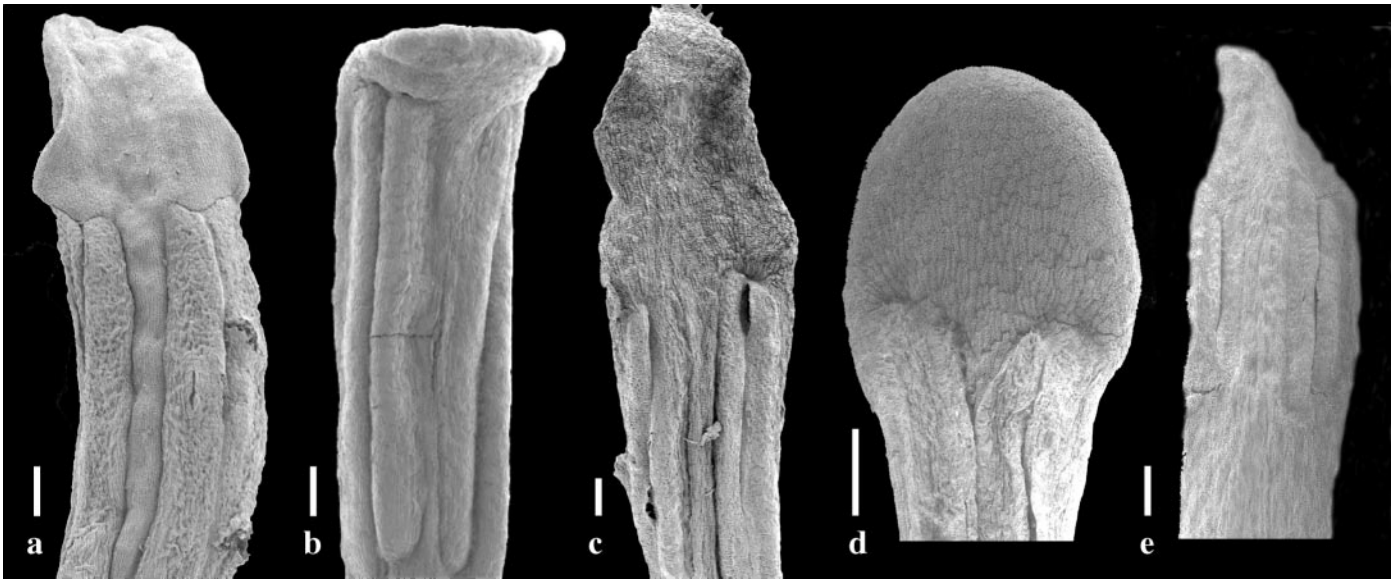

Fig. 15. Stamen morphology in *Anaxagorea*. (a) *A. brachycarpa*: pointed, apex bent away from thecae, lateral extensions of connective absent. (b) *A. dolichocarpa*: truncate, apex bent towards thecae, lateral extensions of connective absent. (c) *A. prinoides*: pointed, apex not bent, lateral extensions of connective absent. (d) *A. rufa*: rounded; apex not bent, lateral extensions of connective absent. (e) *A. acuminata*: pointed, apex bent away from thecae, lateral extensions of connective present. Scale bar = 250 μm.

pointed, as in most of the outgroups and the basal species *A. prinoides*, but there was an early shift to rounded stamens, with some reversals to pointed stamens (Fig. 16a). Truncate stamens originated independently in five species, so this condition is not related to the truncate-peltate state typical of most Annonaceae, which arose in the core group of the family (Doyle and Le Thomas, 1996) and differs significantly in having a peltate cap covering the anthers. The apex is not bent in most *Anaxagorea* species, and this condition is ancestral; bending towards the thecae (*A. brachycarpa* and *A. dolichocarpa)* and away from the thecae (*A. acuminata*, *A. brevipedicellata*, *A. dolichocarpa*, *A. panamensis*, and *A. inundata*) occurred independently in all cases.

In tree B, with *A. pachypetala* in its more basal position, the presence of lateral connective extensions is ancestral in *Anaxagorea* but not homologous with the same state in *Degeneria* and *Galbulimima* (Fig. 17a). The loss of lateral extensions is a synapomorphy of the Central American clade, but it also occurred independently in four other lines. However, in tree A, in which *A. pachypetala* is the sister group of the core South American clade, it is equivocal whether lateral extensions are ancestral in *Anaxagorea* (Fig. 17b). Thus, some of our trees support the view of Walker (1971) that ''leaf-like'' stamens, largely defined by this feature, are primitive in *Anaxagorea*, but in others this is equivocal. It is also equivocal whether the stamens of this type that he observed in *A. phaeocarpa* (identified as *A. costaricensis*) are primitive or secondarily reversed. In neither case are they ancestral for Annonaceae as a whole.

Morphological differentiation of the inner and outer stamens, the former appearing to intergrade with the inner staminodes, is ancestral in *Anaxagorea* (Fig. 16b). Loss of this differentiation is an important synapomorphy linking the Central American and core South American clades, although there are two reversals that unite pairs of species within the core South American clade and another in *A. allenii*.

A striking but problematic character at the level of Annonaceae is the distinction between normal and locellate anthers (character 46). Doyle and Le Thomas (1996) defined this character as referring to mature anthers, but they recognized that septa may be absent at maturity but present early in development, as emphasized by Tsou and Johnson (2003). Tsou and Johnson (2003) also showed that the septa in locellate anthers are of two different types. These are not serious problems for the present study, because *Anaxagorea* does not have locellate anthers, and the two taxa that do have them (*Mkilua*, *Xylopia*) have the same type. However, they indicate that the character needs reassessment in future broader-scale analyses.

As with leaf architectural characters, when stamen characters are removed from the data set, there is an increase in the number of equally parsimonious trees produced, from four to 362. Here, however, there is essentially no change in the consistency and retention indices, from CI = 0.695 to 0.694 and from RI = 0.705 to 0.704. In the strict consensus, *Anaxagorea* is still monophyletic, but the only other clades that remain are the Central American clade, the Asian clade (with internal relationships unresolved), and the clade consisting of *A. angustifolia* and *A. rheophytica*.

Pollen—All pollen characters are taken from Doyle and Le Thomas (1996), without any changes in states or the scoring of outgroups, and with those *Anaxagorea* species scored that have been examined in various studies. As concluded by Doyle and Le Thomas (1996), most pollen characters of *Anaxagorea* have states that are ancestral for Annonaceae and are shared with their closest outgroups in Magnoliales (boat-shaped, monosulcate, single grains with an imperforate tectum and granular infratectum). However, more recent broader-scale analyses indicate that some of these features, most notably granular infratectal sculpture, are derived relative to those of Magnoliaceae, Myristicaceae, and more basal angiosperms (Doyle and Endress, 2000; Sauquet et al., 2003).

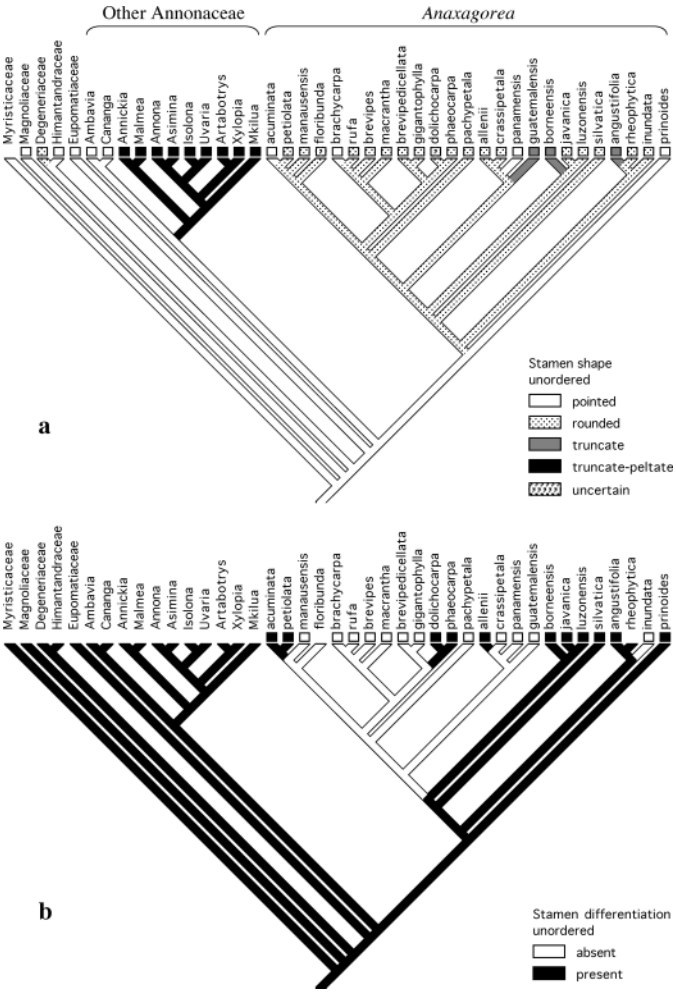

Fig. 16. Tree A, showing inferred evolution of stamen shape (a) and stamen differentiation (b) in *Anaxagorea* and outgroups.

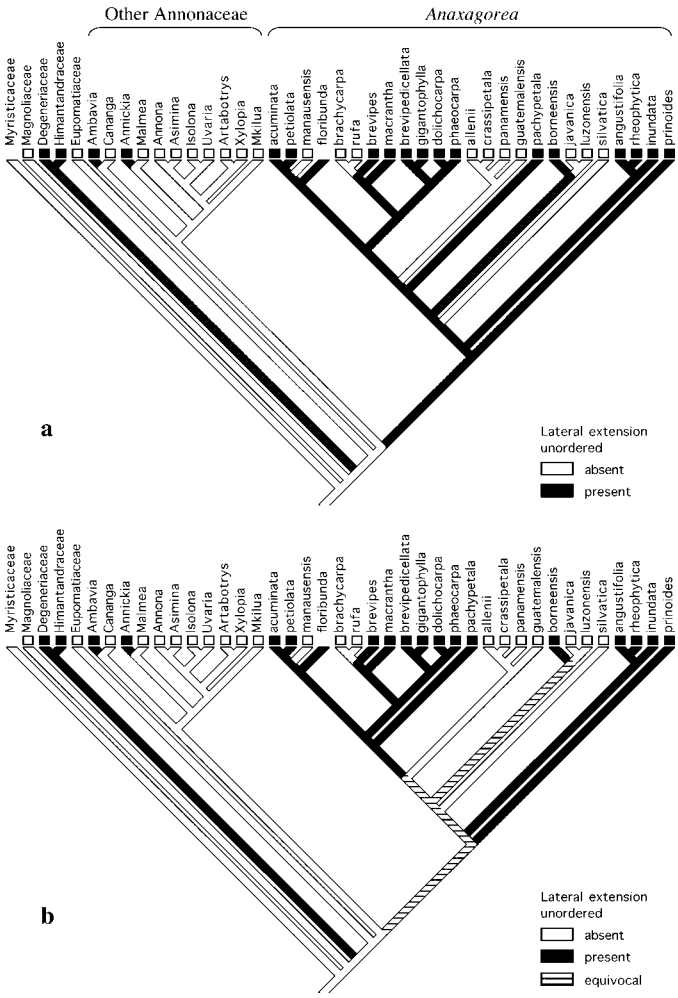

Fig. 17. Inferred evolution of lateral connective extensions on tree B (a) and tree A (b) in *Anaxagorea* and outgroups.

Receptacle and carpels—As noted by Doyle and Le Thomas (1996), shape of the stamen-bearing portion of the receptacle (character 56) and shape of its carpel-bearing apex (character 57) vary independently of each other. We found no variation in either character in *Anaxagorea*, but we included them to assess their status in *Anaxagorea*. As in Doyle and Le Thomas (1996), the flat or convex receptacle apex seen in *Anaxagorea* is a synapomorphy of the Annonaceae, whereas the flat or conical shape of the stamen-bearing portion was retained from lower in Magnoliales.

Detailed information on carpel number (character 58) is available for nearly all species of *Anaxagorea* (Maas and Westra, 1984, 1985); however, there did not seem to be any natural break in the distribution of numbers within the genus. We retained the break at 10 that Doyle and Le Thomas (1996) recognized as potentially useful across Annonaceae, but all species of *Anaxagorea* have more than 10 carpels, which is also the basic state for the rest of the family.

Doyle and Le Thomas (1996) scored stigma shape (character 59) in *Anaxagorea* as sessile, but close examination showed that although most species are indeed sessile, a few are capitate. The ancestral condition in *Anaxagorea* is sessile, and this is a symplesiomorphy shared with other Magnoliales. Capitate stigma shape is an important synapomorphy of the Asian clade but also arose independently in *A. inundata*. Doyle and Le Thomas (1996) inferred that the ancestral state for other Annonaceae was also sessile, but based on our results this state is equivocal. This is because more recent phylogenetic studies (Doyle et al., 2000; Sauquet et al., 2003; Richardson et al., 2004) have shown that *Cananga* (with a capitate stigma) is related to *Ambavia* and other ambavioids and not to *Xylopia* as found by Doyle and Le Thomas (1996).

Ovule number (character 60) does not vary in *Anaxagorea*, but it is an informative character in the family, and the presence of two basal ovules is a conspicuous synapomorphy of *Anaxagorea*.

Fruit—Our treatment of fruit characters follows Doyle and Le Thomas (1996). None of these characters vary in *Anaxagorea*. Recent work indicates that pseudosyncarpy (character 61) arose by different pathways within Annonaceae (Briechle-Mäck, 1994; Svoma, 1998; Chatrou and He, 1999), but because *Annona* is the only pseudosyncarpous member of the family sampled here, this is inconsequential for the present study. We scored the base of the monocarps (character 62) in *Anaxagorea* as stipitate, although the condition differs anatomically from that found in other Annonaceae (Doyle and Le Thomas, 1996). Given present outgroup relationships, it is

equivocal whether the stipitate condition is homologous in *Anaxagorea* and other Annonaceae. Ventral fruit dehiscence (character 64) is a synapomorphy of *Anaxagorea*, not a primitive retention as sometimes thought, whereas the indehiscent state of other Annonaceae is homologous with that in related Magnoliales. The original thickness of the fruit wall in Annonaceae (character 65) was equivocal in Doyle and Le Thomas (1996), but our results indicate that a thin fruit wall was ancestral, as in *Anaxagorea*, due to the different position of *Cananga*.

Seeds—We found no variation in seed characters in *Anaxagorea*, but we retained them in order to consider *Anaxagorea* in the context of other Annonaceae. Character definitions follow Doyle and Le Thomas (1996), with two changes. Doyle and Le Thomas (1996) scored flat and grooved raphe (character 66) as separate states, but with our reduced sampling of Annonaceae we decided to merge the two states, as *Artabotrys* is the only taxon showing a grooved raphe, and it is polymorphic in being flat as well. Changing *Annona* from uncertain (flat or raised), as the *Annona* group was scored by Doyle and Le Thomas (1996), to flat, as a result of exclusion of *Anonidium*, would not affect these results, since *Annona* is nested in a clade with a basically grooved or flat raphe. For similar reasons, in the aril character (70), absent and rudimentary were originally treated as two separate states but have been merged in this study. Johnson and Murray (1995) argued that the bilobed arils of Bocageeae (represented by *Mkilua*) are not homologous with the arils of *Cananga* and *Xylopia*. This is not a problem for the present constrained analyses, in which these three groups are separated, but it means that this character should be reevaluated in future broader-scale analyses.

A micropylar plug (character 67) is absent in *Anaxagorea*, but the presence of a large plug is ancestral in the rest of the family; in the absence of information on outgroups, the polarity of this character is uncertain. The presence of idioblasts in the inner integument (character 71) is a symplesiomorphy of *Anaxagorea* and *Eupomatia*, while the loss of idioblasts is a synapomorphy of the remaining Annonaceae.

The mesotestal fiber character (72) seemed potentially informative in *Anaxagorea*, because Christmann (1986) had reported that some species have longitudinal fibers and others have crossed fibers. The genus was therefore scored as polymorphic by Doyle and Le Thomas (1996). However, we found the crossed arrangement in all *Anaxagorea* species examined. An explanation for this discrepancy is suggested by the fact that we saw only longitudinal fibers in some localized sections of the seed coat, but we observed the crossed arrangement after sectioning in several areas around the seed. Doyle and Le Thomas (1996) concluded that crossed mesotestal fibers were a synapomorphy of the Annonaceae and not homologous with the longitudinal fibers of *Eupomatia*, but with *Eupomatia* as the sister group of Annonaceae mesotestal fibers may be homologous of the two taxa. However, with the present character definition this is equivocal, since the next outgroups have no fibers, and the state in the common ancestor of *Eupomatia* and Annonaceae could be either lack of fibers, longitudinal fibers, or crossed fibers.

Endosperm ruminations of the irregular type (character 73), as in *Anaxagorea*, *Ambavia*, and *Cananga*, are ancestral in Annonaceae and homologous with those in the three closest outgroups. The structure of the ruminations (character 74) and seed shape (character 75) are two new characters in this study. *Anaxagorea* is unusual in having hollow, papery ruminations (Corner, 1949), as opposed to the more common solid ruminations, and this is a synapomorphy for the genus. Similarly, *Anaxagorea* is united by having two markedly asymmetrical seeds, with flat facing surfaces, whereas the seeds in the other Annonaceae are symmetrical.

Conclusion—This study has shown that many of the distinctive features that set *Anaxagorea* apart from other Annonaceae are indeed ancestral and homologous with similar features in other Magnoliales, particularly aspects of leaf architecture, midrib histology, inner staminodes, and stamen morphology. Deviations from these conditions occurred independently within *Anaxagorea* and in other Annonaceae. Some of these homoplastic changes appear to be systematically correlated, such as loss of the adaxial plate in the midrib and a shift from brochidodromous to eucamptodromous venation in *A. silvatica* and the Asian clade and a change from sessile to capitate stigma in the Asian clade. However, these apparent correlations may be misleading, since eucamptodromous venation also originated in several other lines within *Anaxagorea*, and a capitate stigma also arose in *A. inundata*. Similarly, a shift to truncate stamens and loss of inner staminodes both occurred in the Asian clade, but in different species (*A. borneensis*, *A. luzonensis*), and the same changes are seen in different species elsewhere in the genus.

We also identified a greater range of systematically useful variation in some characters than previously documented, although in a few cases apparently promising characters turned out to be uninformative (e.g., the reported variation in mesotestal fiber orientation was contradicted by the finding that all specimens examined had the same state). Many morphological synapomorphies for important clades have also been identified, such as capitate stigma and adaxial cuticle striations in the Asian clade, conical buds and shorter inner petals in the Central American clade, concave secondary veins and stellate trichomes in the core South American clade, foliar astrosclereids in the combined Central American and core South American clade, and numerous inner staminodes with glandular areas in a subgroup of the core South American clade.

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APPENDIX. Character definitions and sources of data. Character numbers and information taken from Doyle and Le Thomas (1996) and Doyle and Endress (2000) are indicated as D&L# and D&E#, respectively, where # is the number of the character in the original study.

Habit

1. Habit (0) tree, (1) liana (D&L1). All *Anaxagorea* species are trees, but this character is variable among other Annonaceae and was retained for consistency in character inclusion.

Midrib histology

2. Midrib histology (0) simple arc, (1) arc with unfused plate, (2) arc with fused plate (D&L5, D&E32).
3. Protuberances of vascular tissue from adaxial midrib plate (0) absent or inconspicuous, (1) prominent.

Sources: Sugiyama (1976a, b, 1979) for outgroups; Koster and Baas (1981) and Sauquet et al. (2003) for Myristicaceae; van Setten and Koek-Noorman (1986) for Annonaceae; and our own observations, in particular for *Anaxagorea*.

Leaf architecture

4. Appearance of midrib on upper side (0) concave or flat, (1) convex (D&L6).
5. Appearance of secondaries on upper side (0) flat-convex, (1) concave.
6. Decurrent base (0) absent, (1) present.
7. Base symmetry (0) symmetrical, (1) asymmetrical.
8. Revolute margin (0) absent, (1) present.
9. Secondary vein pattern (0) strictly brochidodromous, (1) approaching eucamptodromous, (2) pronounced festooned brochidodromous.
10. Secondary vein curvature (0) straight or recurved, (1) moderately curved, (2) strongly curved (ordered) (D&L7).
11. Secondary vein spacing (0) irregular, (1) uniform.
12. Secondary vein angle (0) irregular, (1) uniform, (2) decreasing towards base, (3) increasing toward base. Not scored outside Annonaceae because of uncertain applicability to the festooned brochidromous venation of most outgroups.
13. Number of secondaries on one side (0) less than 10, (1) more than 10.
14. Intersecondaries (0) absent or infrequent, (1) branched (T, Y, or randomly branched), (2) curving downward or unbranched.
15. Tertiary venation (0) reticulate, (1) partially or strongly percurrent (D&L8).

Sources: Based on our own observations of herbarium material and leaf X-rays; P. Maas (Utrecht University, personal communication) for *A. angustifolia*; J. Klackenberg (Swedish Museum of Natural History, personal communication) for *A. macrantha*; Hickey (1973) for terminology.

Leaf anatomy

16. Simple trichomes (0) absent, (1) present (D&L9). Not scored outside *Anaxagorea* because of lack of consistent data.
17. Peltate trichomes (0) absent, (1) present (D&L9).
18. Stellate trichomes (0) absent, (1) present (D&L9).
19. Sclereids in leaves (0) absent, (1) astrosclereids, (2) osteosclereids (D&L10, D&E34).
20. Epidermal crystals (0) druses or absent, (1) solitary rhombic, etc. (D&L11).
21. Oil cells in leaves (0) absent, (1) in sponge parenchyma only, (2) in palisade or both palisade and sponge parenchyma (D&L13, D&E35). Not scored outside Annonaceae because of lack of relevant data.
22. Anticlinal cell walls in unspecialized epidermal cells on abaxial leaf surface (0) distinctly curved, (1) straight to slightly curved.
23. Adaxial striations (0) absent, (1) present.

Sources: Roth (1981), Koek-Noorman and Berendsen (1985), Maas et al. (1986), van Setten and Koek-Noorman (1986).

Wood anatomy

24. Vessel density (0) <10 vessels/mm^2, (1) 10–40 vessels/mm^2, (2) >40 vessels/mm^2 (ordered) (D&L16).
25. Rays (0) narrow, (1) wide (D&L17).

Sources: Vander Wyk and Canright (1956), Maas et al. (1986), Berry et al. (1999).

Inflorescences

26. Inflorescence position (0) terminal (leaf-opposed, supra-axillary, extra-axillary), (1) axillary (D&L22).
27. Flowers (0) solitary (–2), (1) in cymes (D&L23).
28. Upper bract on pedicel (0) caducous, (1) persistent. Not scored outside *Anaxagorea* because of lack of sufficient data.

Sources: Weberling and Hoppe (1996) for outgroups; Maas and Westra (1984, 1985), Maas et al. (1986) for *Anaxagorea* species.

Floral buds

29. Bud shape (0) globose or ovoid, (1) conical. Not scored outside *Anaxagorea* because of excessive variation.

Sources: Maas and Westra (1984, 1985), Maas et al. (1986), our observations.

Corolla

30. Petal whorls (0) >2 cycles, (1) inner and outer whorls, (2) inner whorl absent, (3) petals absent (D&L31, D&E41). Following Doyle and Endress (2000), we scored Himantandraceae as lacking petals: they have petal-like appendages, but these intergrade with stamens and are therefore interpreted as outer staminodes rather than homologues of the petals of other Magnoliales.
31. Petal estivation (outer) in bud (0) imbricate, (1) valvate (D&L32).
32. Relative length of petals (0) equal, (1) inner petals markedly shorter (D&L35).
33. Petal shape (0) oval, (1) elongate, pointed (D&L36).
34. Keel on outer petals (0) absent, (1) present.
35. Keel on inner petals (0) absent, (1) present.

Sources: Characters 30–32, Maas et al. (1986), our observations; character 33, D&L36: Bailey and Smith (1942), Rogstad (1989), Endress (1994b), our observations; characters 34–35: Maas et al. (1986), our observations.

Stamens

36. Outer staminodes (0) absent, (1) present (D&L38).
37. Inner staminodes (0) absent, (1) present (D&L39).
38. Inner staminode number (0) less than 20, (1) more than 20. Not scored outside *Anaxagorea* because of lack of inner staminodes in most other Annonaceae and questionable applicability of the same quantitative limit in other Magnoliales.
39. Inner staminode shape (0) pointed, (1) strictly rounded, (2) always or sometimes truncate.
40. Glandular area on inner staminodes (0) absent, (1) present.
41. Stamen number (0) less than 100, (1) more than 100. Not scored outside *Anaxagorea* because of sporadic data and questionable validity of the same break between states.
42. Stamen apex (0) pointed, (1) rounded, (2) truncate, (3) truncate-peltate (D&L40). Following arguments in Doyle et al. (2000), we eliminated the peltate-apiculate state in D&L40 and rescored *Cananga* as pointed, *Xylopia* as truncate-peltate.
43. Apical flexure of connective (0) absent, (1) bent abaxially (toward thecae), (2) bent adaxially (away from thecae). Not scored outside *Anaxagorea* because the distinction cannot be applied to the peltate-truncate stamens of most other Annonaceae and reliable data are not available for other Magnoliales.
44. Lateral extension of connective (0) absent, (1) present.
45. Differentiation between inner and outer stamens (0) absent, (1) present. Not scored outside *Anaxagorea*.
46. Anthers (0) normal, (1) locellate (at maturity) (D&L41).

Sources: Le Thomas (1969), Endress (1984), Maas et al. (1986), Endress and Hufford (1989), Endress (1994a), our observations.

Pollen

47. Pollen unit (0) single (monads), (1) compound (tetrads, polyads) (D&L42).
48. Proximal exine (0) normal, (1) reduced (D&L43).
49. Aperture (0) sulcate, (1) inaperturate, (2) sulculate (D&L44).
50. Pollen size (average) (0) small (<45 μm), (1) medium, (2) large (>90 μm) (ordered) (D&L45).
51. Pollen shape (0) elongate (boat-shaped), (1) globose (D&L46).
52. Tectum (0) imperforate (or with very small perforations), (1) verrucate, (2) reticulate-perforate (D&L47).
53. Infratectal structure (0) granular, (1) intermediate-columellar (D&L49).
54. Nexine foliations (0) absent, (1) 1–2, often discontinuous, (2) multiple, continuous, often contorted (D&L51). This character was ordered in

Doyle and Le Thomas (1996), but following arguments in Doyle et al. (2000), we have redefined it as unordered.

55. Outer foliations (0) undifferentiated, (1) thickened (D&L52).

Sources: Le Thomas and Lugardon in Maas and Westra (1984), Hesse et al. (1985) for *Anaxagorea*.

Receptacle

56. Stamen-bearing portion of receptacle (0) flat or conical, (1) globose or short cylindrical, (2) elongate (D&L53).
57. Apex of receptacle (carpel-bearing portion) (0) elongate, (1) flat or convex, (2) concave (D&L54).

Sources: van Heusden (1992); our observations for *Anaxagorea*.

Carpels

58. Average number of carpels (0) 1 (rarely 2), (1) 2–10, (2) more than 10 (D&L55).
59. Stigma (0) sessile, (1) capitate (base strongly constricted), (2) on more or less elongate style (D&L56).
60. Ovule number (0) numerous, (1) two lateral, (2) two basal, (3) one basal (D&L57).

Sources: Hesse et al. (1985), Morawetz and Le Thomas (1988), van Heusden (1992), van Setten and Koek-Noorman (1992), Berry et al. (1999), our observations.

Fruit

61. Fruit (0) apocarpous, (1) pseudosyncarpous (at least basally), (2) parasyncarpous (D&L58).
62. Base of monocarps (0) sessile or nearly so, (1) stipitate (D&L60).
63. Articulation of stipe (0) basal, (1) apical (D&L61).
64. Fruit dehiscence (0) ventrally dehiscent, (1) indehiscent, (2) dorsally dehiscent (D&L62).
65. Fruit wall (0) thick (>1 mm, generally woody or leathery), (1) thin (<1 mm, generally juicy) (D&L63).

Sources: van Setten and Koek-Noorman (1992) for *Anaxagorea*.

Seeds

66. Raphe (0) more or less pronounced groove or flat, (1) more or less pronounced rib (D&L65).
67. Micropylar plug (0) absent or small, (1) large (D&L66).
68. Endosperm (0) normal, (1) glass-like or stony (D&L67).
69. Colored oil cells in endosperm (0) absent, (1) present (D&L68).
70. Aril (0) absent or rudimentary, (1) bilobed (D&L70).
71. Idioblasts (oil cells) in seed coat (0) in inner integument, (1) absent, (2) in outer integument, (3) in nucellus (D&L73).
72. Mesotestal fibers (0) absent, (1) longitudinal, (2) crossed, (3) transverse (D&L74).
73. Ruminations (0) absent, (1) thick, irregular plates or pegs, (2) spiniform, (3) lamelliform (including dissected but thin plates) (D&L75). Myristicaceae scored as unknown because their ruminations are not testal (Sauquet et al., 2003).
74. Rumination structure (0) hollow, (1) solid. Myristicaceae not scored because of their non-testal ruminations.
75. Seed shape (0) asymmetrical, (1) symmetrical.

Sources: Corner (1949); Periasamy and Swamy (1961); Christmann (1986) for *A. brevipes*, *A. prinoides*, *A. silvatica*, and *A. luzonensis*; van Setten and Koek-Noorman (1992); Garwood (1995); Svoma (1997); Doyle and Endress (2000); our observations for mesotestal fibers, ruminations, and seed shape in *Anaxagorea*.